Univ. of
Toronto
Library

BINDING LIST JAN 16 1928

Digitized by the Internet Archive
in 2010 with funding from
University of Toronto

http://www.archive.org/details/annalsoftropical05live

Annals of Tropical Medicine and Parasitology

THE UNIVERSITY OF LIVERPOOL

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

VOLUME V

(April 20, 1911, to February 26, 1912)

With twenty-five plates, thirty-six figures in text, fifty-seven charts, and three maps

21094
14.12.27

LIVERPOOL:
AT THE UNIVERSITY PRESS, 57 ASHTON STREET.

RC
960
A55
v.5

CONTENTS

No. 1

PAGE

McCarrison, Robert.

Further Experimental Researches on the Etiology of Endemic Goitre. Plates I, II. 1

Carter, Captain R. Markham.

Non-ulcerating Oriental Sore : the Cultural Characteristics of the Parasite as compared with a New similar Parasite in *Erthesina fullo* (Thumb), a Pentatomid Bug. Plates III, IV 15

Critien, A.

Infantile Leishmaniasis (Marda tal Biccia) in Malta. 37

Thomson, David.

I.—A Research into the Production, Life and Death of Crescents in Malignant Tertian Malaria, in Treated and Untreated Cases, by an Enumerative Method 57

II.—The Leucocytes in Malarial Fever : a Method of Diagnosing Malaria long after it is apparently cured 83

Boyce, Sir Rubert.

Note upon Yellow Fever in the Black Race and its bearing upon the Question of the Endemicity of Yellow Fever in West Africa. Two diagrams ... 103

Fantham, H. B.

On the Amoebae Parasitic in the Human Intestine, with Remarks on the Life-Cycle of *Entamoeba coli* in Cultures 111

Newstead, Robert.

Some Further Observations on the Tsetse-fly, described in these Annals as *Glossina grossa*, etc. 125

Korke, Vishnu T.

On the Correlation between Trypanosomes, Leucocytes, Coagulation Time, Haemoglobin and Specific Gravity of Blood 127

CONTENTS

No. 2

PAGE

In Memoriam—Professor Sir Rubert Boyce, F.R.S. With portrait 133

GABBI, PROFESSOR UMBERTO.

Note on Tropical Diseases in Southern Italy 135

NEWSTEAD, R.

The Papataci Flies (*Phlebotomus*) of the Maltese Islands. Plates V-VII ... 139

MCCARRISON, ROBERT.

The Experimental Transmission of Goitre from Man to Animals. Plates VIII-X 187

NAUSS, RALPH W.; and YORKE, WARRINGTON.

Reducing Action of Trypanosomes on Haemoglobin 199

STEPHENS, J. W. W.

The Anti-Malarial Operations at Ismailia. Two Maps 215

NEWSTEAD, R.; and CARTER, HENRY F.

On some New Species of African Mosquitos (Culicidae). Plate XI 233

TODD, JOHN L.; and WOLBACH, S. B.

The Diagnosis and Distribution of Human Trypanosomiasis in the Colony and Protectorate of the Gambia. One Map 245

YORKE, WARRINGTON; and NAUSS, RALPH W.

The Mechanism of the Production of Suppression of Urine in Blackwater Fever. Plates XII-XIII 287

SIMPSON, G. C. E.; and EDIE, E. S.

On the Relation of the Organic Phosphorus Content of Various Diets to Diseases of Nutrition, particularly Beri-Beri 313

CONTENTS

No. 3

Ross, Professor Sir Ronald ; and Stott, Walter. PAGE

Tables of Statistical Error 347

Seidelin, Dr. Harald.

Notes on some Blood Parasites in Reptiles. Plates XIV-XV 371

Ross, Sir Ronald ; and Edie, E. S.

Some experiments on Larvicides 385

Graham, W. M.

An Investigation of the Effects produced upon the Excretion of Urinary Pigments by Salts of Quinine. Plate XVI 391

Yorke, Warrington.

The Passage of Haemoglobin through the Kidneys. Plate XVII 401

Ross, Sir Ronald ; and Thomson, David.

Pseudo-Relapses in Cases of Malarial Fever during Continuous Quinine Treatment 409

Yorke, Warrington ; and Blacklock, B.

The Trypanosomes found in Two Horses Naturally Infected in the Gambia. Plate XVIII 413

Wise, K. S.

An Examination of the City of Georgetown, British Guiana, for the Breeding Places of Mosquitos 435

Stannus, Hugh S. ; and Yorke, Warrington.

A Case of Human Trypanosomiasis in Nyasaland with a Note on the Pathogenic Agent. Plate XIX 443

McCarrison, Robert.

A Second Series of Experiments dealing with the Transmission of Goitre from Man to Animals. Plates XX-XXII 453

Thomson, David.

A New Blood-Counting Pipette, for estimating the numbers of Leucocytes and Blood Parasites per cubic millimetre 471

Fantham, H. B.

Some Researches on the Life-Cycle of Spirochaetes 479

Stephens, J. W. W.

Desmogonius desmogonius, a New Species and Genus of Monostome Flukes. Plate XXIII 497

CONTENTS

No. 4

PAGE

SEIDELIN, DR. HARALD

Notes on some Blood-Parasites in Man and Mammals. Plate XXIV ... 501

PATTON, CAPTAIN W. S. ; and CRAGG, CAPTAIN F. W.

The Genus *Pristirhynchomyia*, Brunetti, 1910. Plate XXV 509

PATTON, CAPTAIN W. S. ; and CRAGG, CAPTAIN F. W.

The Life History of *Philaematomyia insignis*, Austen 515

BLACKLOCK, B.

The Measurements of a Thousand Examples of *Trypanosoma vivax* ... 521

THOMSON, JOHN GORDON.

Enumerative Studies on *T. brucei* in Rats and Guinea-Pigs, and a Comparison with *T. rhodesiense* and *T. gambiense* 531

BLACKLOCK, B.

A Note on the Measurements of *Trypanosoma vivax* in Rabbits and White Rats 537

ROSS, SIR RONALD ; and THOMSON, DAVID.

A Case of Malarial Fever, showing a True Parasitic Relapse, during Vigorous and Continuous Quinine Treatment. 539

INDEX

INDEX

PAGE

INDEX OF AUTHORS iii
GENERAL INDEX iii
INDEX OF GENERA, SPECIES AND VARIETIES NEW TO SCIENCE vii

INDEX OF AUTHORS

PAGE

Blacklock, B. 521, 537
Blacklock, B. ; and Yorke, W. 413
Boyce, Sir Rubert 103
Carter, H. F. ; Newstead, R. 233
Carter, R. Markham 15
Cragg, F. W. ; and Patton, W. S., 509, 515
Critien, A. 37
Edie, E. S. ; and Ross, Sir R. 385
Edie, E. S. ; and Simpson, G. C. E. 313
Fantham, H. B. 111, 479
Gabbi, Umberto 135
Graham, W. M. 391
Korke, Vishnu T. 127
McCarrison, Robert 1, 187, 453
Nauss, R. W. ; and Yorke, W. 199, 287
Newstead, Robert 125, 139
Newstead, R. ; and Carter, H. F. 233
Patton, W. S. ; and Cragg, F. W., 509, 515

PAGE

Ross, Sir R. ; and Edie, E. S. 385
Ross, Sir R. ; and Stott, W. 347
Ross, Sir R. ; and Thomson, D. ... 409, 539
Seidelin, Harald 371, 501
Simpson, G. C. E. ; and Edie, E. S. 313
Stannus, H. S. ; and Yorke, W. 443
Stephens, J. W. W. 215, 497
Stott, W. ; and Ross, R. 347
Thomson, David 57, 83, 471
Thomson, D. ; and Ross, Sir R. ... 409, 539
Thomson, J. G. 531
Todd, J. L. ; and Wolbach, S. B. 245
Wise, K. S. 435
Wolbach, S. B. ; and Todd, J. L. 245
Yorke, Warrington 401
Yorke, W. ; and Blacklock, B. 413
Yorke, W. ; and Nauss, R. W. ... 199, 287
Yorke, W. ; and Stannus, H. S. 443

GENERAL INDEX

PAGE

Amoebae parasitic in the human intestine 111
Amoeboid parasites in blood-plasma of Lizard 380
Anophelines, Anti-Larval measures at Ismailia 220
,, Breeding places of, at Ismailia 221
Argus persicus, Spirochaetes in 485
Auto-agglutination in human trypanosomiasis 260
Bacillus bulgaricus in the treatment of goitre 12
Barbados, Yellow Fever in 106
Beri-Beri, Phosphorus, Organic, content of diet and 313
,, Rice, polished and unpolished in ..... 314 *et seq.*
Blacklock, B., Measurements of *T. vivax* 521, 537

PAGE

Blacklock, B., and Yorke, W., Trypanosomes of naturally infected horses 413
Blackwater Fever, Kidney in ... 402 *et seq.*
,, ,, Leucocytes in 93
,, ,, Urine, Mechanism of Production of suppression of, in 287
Blood-counting Pipette 471
Blood, in Trypanosome infections, 127, 200
,, Reducing action of Trypanosomes on haemoglobin 200
Boyce, Sir R. In Memoriam 133
Boyce, Sir R. Yellow fever in the Black Race 103
Cancrum oris in Infantile Leishmaniasis 39
Carter, H. F., and Newstead, R. New African Mosquitos 233

PAGE

Carter, R. M. Non-ulcerating oriental sore; cultural characteristics of parasite 15
Cellia arnoldi 238
,, *pseudosquamosa*, n. sp. 236
,, *squamosa*, var. *arnoldi* 238
Cragg, F. W., and Patton, W. S. The Genus *Pristirhynchomyia* 509
Cragg, F. W., and Patton, W. S. Life history of *Philaematomyia insignis*... 515
Critien, A. Infantile Leishmaniasis in Malta 37
Dengue Fever in Italy 137
Desmogonius desmogonius, n. g., n. sp. 497
Dysentery, Amoebae of 111
Edie, E. S., and Ross, Sir R. Experiments on larvicides 385
Edie, E. S., and Simpson, G. C. E. Organic phosphorus content of diet and Beri-Beri 313
Entamoeba sp. (Noc) 113
,, *coli* 112, 116, 118
,, ,, Life-cycle of, in cultures 119
,, ,, Leucin, Skatol and Tyrosin in Culture media of 120
,, *hominis* 113, 116, 118
,, *histolytica* 113, 116, 118
,, *minuta* 115, 116, 118
,, *nipponica* 115, 116, 118
,, *phagocytoides* 114, 116, 119
,, *tetragena* 114, 116, 118
,, *tropicalis* 112, 116, 118
,, *undulans* 115
Enumerative Methods 474
,, ,, Blood-counting Pipette 471
,, Studies of crescents in malignant tertian malaria 57
,, Studies on *T. brucei* ... 531
Erthesina fullo 23
,, ,, Parasites from intestinal tract of 24
Fantham, H. B. On the Amoebae parasitic in the human intestine... 111
Fantham, H. B. Life-cycle of Spirochaetes 479
Filaria imperatoris 371

PAGE

Gabbi, U. Tropical diseases in Southern Italy 135
Gambia, Horses naturally infected with Trypanosomes in 413
Georgetown, Mosquito breeding places in 435
Gland palpation and puncture in human trypanosomiasis ..... 257 *et seq.*
Glossina grossa, Synonym of *Gl. nigrofusca* 125
,, *morsitans*, *T. rhodesiense* probably transmitted by... 449
,, *nigrofusca*, n. sp. 125
,, *palpalis*, var. *Wellmani* 125
Goitre, Earthworms in the spread of... 187
,, Endemic, Experimental researches on Etiology 1
,, ,, treatment of 12
,, Experimental, Carbonates of Lime, Magnesium, and Sodium, in... 458 *et seq.*
,, ,, transmission from man to animals, 187, 453
Graham, W. M. Salts of quinine and the excretion of urinary pigments... 391
Haemoglobin, Reducing action of Trypanosomes on 199
Haemoglobinuria, Experimental 404
,, ,, and Suppression of urine 287
Haemogregarina imperatoris, n.sp. 374
Ismailia, Anopheline breeding places 221
,, Anti-malarial operations at 215
Italy, Southern, Tropical diseases of... 135
Kala-azar, Infantile 11
,, ,, in Italy 136
Kidneys, Blackwater Fever, suppression of urine in ..... 402 *et seq.*
,, Haemoglobin, passage of, through 401
,, *Piroplasma canis* 403, 406
Korke, V. T. Correlation between trypanosomes, leucocytes, coagulation time, haemoglobin and specific gravity of blood 127
Lactic ferments in the treatment of goitre 12

PAGE

Larvicides..... 385
Leishmania infantum in infantile Leishmaniasis 44, 136
Leishmaniasis, Infantile, Cancrum oris in ... 39
,, ,, clinical picture 38
,, ,, in Italy..... 136
,, ,, in Malta ... 37
Leucocyte counts in blackwater fever 93
,, ,, malaria 65, 83
,, ,, oriental sore ... 20
Leucocytes ingest malarial parasites, 72, 85
Leucocytic extracts in Malaria 86
McCarrison, R. Experimental researches on the Etiology of Endemic Goitre 1
McCarrison, R. Experimental transmission of Goitre..... 187, 453
Malaria, Diagnosis by leucocytes..... 83
,, Ismailia, anti-malarial operations at 215
,, Latent, diagnosis of 89
,, Leucocyte counts in 65
,, Leucocytes in 83
,, Leucocytic extracts in 86
,, Pilocarpine injections in..... 87
,, Quinine and excretion of urinary pigments 391
,, Quinine Prophylaxis.....228, 398
,, Quinine Prophylaxis and cure 73
,, Quinine treatment, pseudo-relapses during 409
,, Quinine treatment, true parasitic relapse during 539
,, Relapses, pseudo, during quinine treatment 409
,, Relapses, true parasitic, during quinine treatment ... 539
,, Yucatán 507
Malarial parasites, ingested by leucocytes 72, 85
,, ,, Methylene Blue, the effect of, on Crescents..... 67
,, ,, Production, life and death of crescents 57
,, ,, Quinine, effect of, on crescent production..... 66
Malta, Infantile Leishmaniasis in..... 37

PAGE

Mediterranean Fever in Italy 135
Mercuric chloride as larvicide 388
Methylene blue, the effect of, on malarial crescents 67
Mikrofilaria. See *Filaria*.
Mosquitos, African, new species of ... 233
,, Breeding places in Georgetown 435
,, Larvicides 385
Molluscs, Lamellibranch, Spirochaetes of.....488 *et seq.*
Nauss, R. W., and Yorke, W. Action of trypanosomes on haemoglobin... 199
Nauss, R. W., and Yorke, W. Suppression of urine in Blackwater fever 287
Neocellia christyi, n. sp..... 238
Newstead, R. Papataci Flies of Maltese Islands 139
Newstead, R. The Tsetse-fly described as *Glossina grossa*, etc... 125
Newstead, R., and Carter, H. F. New African Mosquitos 233
Nyasaland, Trypanosomiasis, human, cases of, in 443
Oriental Sore, Italy 137
,, ,, non-ulcerating, cultural characteristics of parasite of 15
Ornithodorus moubata, Spirochaetes in 485
Papataci Fever in Italy 137
,, Flies of Maltese Islands ... 139
Paraplasma subflavigenum, n. sp. 504
Patton, W. S., and Cragg, F. W. The Genus *Pristirhynchomyia* 509
Patton, W. S., and Cragg, F. W. Life history of *Philaematomyia insignis*... 515
Petroleum as larvicide 385
Philaematomyia gurnei, n. sp. 513
,, *insignis*, Life history of 515
,, *lineata* 512
Phlebotomus, Bionomics of ...140 *et seq.*
,, Morphology of Genus 153 *et seq.*
,, Prophylactic Measures 148 *et seq.*
Phlebotomus minutus 169
,, *nigerrimus*, n. sp. 168
,, *papatasii* 174
,, *perniciosus*, n. sp. 172
Pilocarpine injections in Malaria..... 87

PAGE

Piroplasma canis, Kidney in ...403, 406
Plasmodium sp., monkey naturally infected with 505
,, *falciparum*, Biology of sexual form of..... 57
Polyneuritis, Experimental, in animals 314 *et seq.*
,, ,, yeast, Protective and curative effect of, in..... 321 *et seq.*
Potassium cyanide as larvicide 388
Pristirhynchomyia, Genus 509
Pyretophorus distinctus, n. sp..... 234
,, ,, var. *melanocosta*, n. var.... 236
Quinine, Effect of, on crescent production 66
,, Malaria, pseudo-relapses in, during treatment with ... 409
,, Malarial Prophylaxis, 73, 228, 398
,, Urinary Pigments, Effect produced upon excretion of, by 391
Reedomyia simulans, n. sp. 240
Reptiles, Blood parasites of 371
Ross, Sir R., and Edie, E. S. Experiments on larvicides 385
Ross, Sir R., and Stott, W. Tables of Statistical error 347
Ross, Sir R., and Thomson, D. Pseudo-Relapses in malaria during quinine treatment 409
Ross, Sir R., and Thomson, D. True parasitic relapse in Malarial Fever during quinine treatment... 539
Sanitas-Okol as larvicide 386
Schaumann, H. Beri-Beri ...313 *et seq.*
Seidelin, H. Blood parasites in man and mammals 501
Seidelin, H. Blood parasites in reptiles 371
Simpson, G. C. E., and Edie, E. S. Organic phosphorus content of diet and Beri-Beri 313
Sleeping Sickness. See Trypanosomiasis, Human.
Spirochaeta anodontae, Spores of 489
,, *balbianii*, Spores of 489

PAGE

Spirochaeta duttoni, Life-cycle of, 480 *et seq.*
,, *marchouxi*, Life-cycle of 480 *et seq.*
,, *recurrentis*, Life-cycle of... 480 *et seq.*
,, *solenis*, n. sp.....480, 488, 493
Spirochaetes, Life-cycle of 479
Stannus, H. S., and Yorke, W. Case of human trypanosomiasis in Nyasaland 443
Statistical Error, Tables of 347
Stephens, J. W. W. Anti-malarial operations at Ismailia 215
Stephens, J. W. W. New species and genus of monostome flukes..... 497
Stott, W., and Ross, Sir R. Tables of Statistical Error 347
Strychnine as larvicide 389
Suez-Canal, Anti-malarial operations at Ismailia 215
Thomson, D. Blood-counting Pipette 471
Thomson, D. Leucocytes in malarial fever; method of diagnosing malaria 83
Thomson, D. The production, life, and death of crescents in malignant tertian malaria..... 57
Thomson, D., and Ross, Sir R. Pseudo-Relapses in malaria during quinine treatment 409
Thomson, D., and Ross, Sir R. True parasitic relapse in Malarial Fever during quinine treatment 539
Thomson, J. G. Enumerative studies on *T. brucei* 531
Thyroid gland, Goitre .....4 *et seq.*
,, ,, ,, Experimental 189 *et seq.*
454 *et seq.*
Todd, J. L., and Wolbach, S. B. Trypanosomiasis in the Gambia ... 245
Trematodes, *Desmogonius desmogonius*, n.g., n.sp. 497
T. brucei, Enumerative studies on ... 531
T. dimorphon .....415 *et seq.*
T. lewisi, Rats naturally infected with 506
T. rhodesiense in Nyasaland 443
T. vivax .....414 *et seq.*
T. ,, Measurements of 521, 537

PAGE

Trypanosomes, Correlation between Trypanosomes, Leucocytes, coagulation time, haemoglobin and specific gravity of blood 127
Trypanosomes, Horses naturally infected with Tryps. ... 413
,, Reducing action of, on haemoglobin..... 199
Trypanosomiasis, Human, Auto-agglutination..... 260
,, ,, Case of, in Nyasaland 443
,, ,, Diagnosis and distribution in the Gambia ... 245
,, ,, Gland palpation and puncture 257 *et seq.*
,, ,, Immunity 282
,, ,, Preventive measures for the Gambia ... 283 *et seq.*
,, ,, Pulse and Temperature 273
Tsetse-flies, *Glossina grossa* synonym of *Gl. nigrofusca* 125
,, ,, *nigrofusca*, n. sp. 125
,, ,, *palpalis*, var. *Wellmani* 125
Urinary Pigments, Quinine and excretion of 391
West Africa, Yellow Fever in 103
Wise, K. S. Mosquito Breeding-places in Georgetown..... 435
Wolbach, S. B., and Todd, J. L. Trypanosomiasis in the Gambia ... 245
Yeast, Protective and curative effect of, in experimental polyneuritis... 321 *et seq.*
Yellow Fever, Barbados 106
,, ,, Black Race 103
,, ,, Endemicity in West Africa 103
,, ,, Immunity .....104 *et seq.*
Yorke, W. Passage of haemoglobin through the Kidneys 401
Yorke, W., and Blacklock, B. Trypanosomes of naturally infected horses 413
Yorke, W., and Nauss, R. W. Action of trypanosomes on haemoglobin... 199
Yorke, W., and Nauss, R. W. Suppression of urine in Blackwater fever 287
Yorke, W., and Stannus, H. S. Case of human trypanosomiasis in Nyasaland 443
Yucatán, Malaria in 507

INDEX OF GENERA, SPECIES AND VARIETIES NEW TO SCIENCE

PAGE

Cellia pseudosquamosa 236
Desmogonius 497
,, *desmogonius* 497
Glossina nigrofusca 125
Haemogregarina imperatoris..... 374
Neocellia christyi 238
Paraplasma subflavigenum 504
Philaematomyia gurnei 513
Phlebotomus nigerrimus 168
,, *perniciosus* 172
Pyretophorus distinctus 234
,, ,, var. *melanocosta*, 236
Spirochaeta solenis 480, 488, 493
Reedomyia simulans 240

Volume V April, 1911 No. 1

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY

THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Editor

PROFESSOR RONALD ROSS, MAJOR I.M.S. (RET.), D.P.H., F.R.C.S., D.Sc., LL.D., F.R.S., C.B.

In Collaboration with

J. W. W. STEPHENS, M.D., CANTAB., D.P.H.
R. NEWSTEAD, M.Sc., A.L.S., F.E.S., Hon. F.R.H.S.
J. L. TODD, B.A., M.D., C.M. McGill, D.Sc., Liv.
H. WOLFERSTAN THOMAS, M.D., C.M. McGill.
ANTON BREINL, M.U.Dr.
PROFESSOR SIR RUBERT BOYCE, M.B., F.R.S.

C. Tinling & Co., Ltd.
Printers to the University Press of Liverpool
53 Victoria Street



Yours truly
David Bruce

FURTHER EXPERIMENTAL RESEARCHES ON THE ETIOLOGY OF ENDEMIC GOITRE*

BY

ROBERT McCARRISON, M.D., R.U.I., M.R.C.P (LOND.), I.M.S.

AGENCY SURGEON, GILGIT

(*Received for publication* 25 *November*, 1910)

OBJECT OF THE RESEARCH

In a communication which I had the honour to make to the Royal Society, on the 26th November, 1908, I described the results of my experimental researches on the etiology of goitre up to that date, as follows:—

1. Goitre can be experimentally produced in man by the administration of the matter in suspension separated by filtration from waters which are known to be goitre-producing.

2. Goitre cannot be so produced when the suspended matter is boiled.

3. The disease is due, therefore, not to the mineral but to the living component of the suspended matter, in other words, to a living organism of disease.

4. The incubation period of experimentally produced goitre is thirteen to fifteen days.

5. Goitre can be cured by the administration of intestinal antiseptics. It is possible, therefore, that the organism which is the cause of the disease is parasitic in the human intestine.

These conclusions were based on the results of experimental observations carried out on man during the years 1907-1908. It has been the object of the present research to test their accuracy by

*Read in abstract before the Royal Society, 2 February, 1911.

experimentation on a larger number of men. My investigations may be summarised as follows:—

I. The experimental administration to man of suspended matter, separated by filtration and sedimentation from goitre-producing waters.

II. The experimental administration to man of suspended matter from the same source, which had been subjected to *boiling* for at least ten minutes.

III. The effect of the administration of filtered goitre-producing water to man under experimental conditions.

IV. The action exercised by the lactic ferments when applied to the treatment of goitre.

V. The effect on dogs of the administration of extracts from faeces of goitrous individuals.

I and II. THE EXPERIMENTAL ADMINISTRATION TO MAN OF THE *(a)* UNTREATED AND *(b)* BOILED SUSPENDED MATTER OF GOITRE-PRODUCING WATERS

Experiment G.—Carried out during October and November, 1909.

Thirteen healthy young men, aged between eighteen and twenty-four years, volunteered for this experiment. All were new-comers to the district, and were in every respect perfectly normal. During the whole course of the experiment the men lived under the strictest guard. Their diet was chiefly vegetable. They were kept at hard exercise, and were not permitted to handle the soil, or to drink or eat anything whatsoever except what was prescribed. The hygienic conditions of life under which they lived were excellent. They were encamped on a non-goitrous site, and were provided with water for drinking, bathing, and other domestic purposes, from the Bermis spring, which is non-goitre producing, but which, as an additional precaution, was boiled. In no case was the restraint imposed upon them broken through.

Kashrote Village water was used for the purposes of this experiment. Goitre prevails in this village to the extent of 45 per cent. The water flows through a narrow irrigation channel, which receives the drainings from cultivated land, and is subjected to much contamination both from human and animal sources. This

water, which at the time of collection was purposely made muddy by agitation, was brought daily from the village to the camp on the hilly ground above, a distance of about one and a half miles. It was allowed to stand in suitable vessels and to deposit its sediment. Four ounces of this sediment were administered to each subject before the morning and evening meals for thirty days.

The results were as follows:—

1. Nine individuals showed no change in the thyroid gland which could be detected by physical examination.

2. In two other cases a uniform swelling of the organ was observed on the tenth day of the experiment. The swelling gave rise to feelings of discomfort, and complaint was made of throbbing in the neck and the tightness of collar bands which had previously fitted well. The measurement of the neck increased by one centimetre in one case, by one and a half in the other. The enlargement persisted up to the twentieth day of observation and then gradually disappeared; the gland was observed to have regained its normal size on the thirtieth day, when the experiment was concluded.

3. In the two remaining cases a more marked reaction on the part of the thyroid gland was observed. In both these cases the right lobe was chiefly affected, and the swelling was accompanied with feelings of discomfort, throbbing in the neck and tightness of the neck band. The measurement of the neck increased one and a half centimetres in one case, and slightly over one centimetre in the other. The enlargement made its appearance between the tenth and fifteenth days of the experiment in each of these cases, and persisted up to the thirtieth day, when the experiment was terminated.

Unfortunately, no photographic records of these cases could be obtained owing to the fact that I had just returned from Europe, and my materials for obtaining such records had not reached me. The thyroid enlargements observed in the two latter cases resembled very closely that shown in Figs. 2 and 9.

Experiment H.—Carried out during October and November, 1909.

The conditions of this experiment were precisely similar to those detailed in the previous one. In this case, however, the thirteen individuals who volunteered for it were given the same

Kashrote water sediment *which had been previously boiled for ten minutes*. In none of these individuals could any reaction on the part of the thyroid gland be detected. The experiment, which lasted thirty days, was carried out concurrently with Experiment G, and formed a very suitable control to it.

EXPERIMENT I.—Carried out from 15th March to 9th May, 1910.

The conditions of this experiment were precisely similar to those of G and H, with the exception that in this case the goitre-producing water of Kashrote was filtered through a Berkefeld house-filter. The deposit on the candle was washed off in distilled water, and a quantity of this dark grey mixture, equal to about two ounces, was given to each subject in milk before the morning and evening meals. Ten individuals volunteered for this experiment. Their average age was twenty-two. All were enjoying excellent health and were in every respect normal.

Of these individuals four showed no reaction whatever on the part of the thyroid gland during the fifty-five days the experiment lasted. One developed a slight degree of enteritis on the fifteenth day when the experiment in his case was discontinued. The following is the more detailed account of the alterations observed in the remaining five cases:—

1. G.M.—15.3.—The thyroid gland was normal. The measurement of the neck on this date, when the experiment commenced, was thirty-three and a half centimetres. The subject was photographed. (Fig. 1.)

31.3—The measurement of the neck was thirty-four and a half centimetres. The right lobe of the thyroid gland was slightly enlarged. There was distinct fullness of the neck on that side. During the act of deglutition the right lobe of the organ was felt to pass under the fingers as a rounded boss. The subject complained of tightness of the shirt collar and of throbbing in the neck.

6.4—There was no marked change to be recorded. The subject complained of the same symptoms as at the previous examination. The outline of the gland was readily seen and felt. The measurement of the neck was thirty-four centimetres.

10.4—There was no appreciable change on physical examination. The measurement of the neck was thirty-four and a half centimetres. The subject complained of inability to button his collar band.

15.4—The thyroid gland was noticeably enlarged. The enlargement was especially well seen during the act of swallowing. The measurement of the neck was thirty-five centimetres. The subject was photographed. (Fig. 2.)

21.4—The measurement of the neck was thirty-four and a half centimetres.

1.5—The thyroid enlargement was not so evident. The neck measured thirty-four centimetres.

9.5—The thyroid gland was undoubtedly smaller on this date than on the 15th April. The measurement of the neck was thirty-four centimetres. The experiment was discontinued and the subject was given thymol, ten grains night and morning. No trace of enlargement of the thyroid gland was observable one month later.

2. S.A.—15.3.—The thyroid gland was normal. The measurement of the neck was thirty-three centimetres. The subject was photographed. (Fig. 3.)

31.3.—There was slight enlargement of the left lobe of the thyroid gland which was clearly seen and readily felt when the subject swallowed. The measurement of the neck was thirty-three and a half centimetres.

6.4.—The thyroid gland was noticeably enlarged especially on the left side. The enlargement was well seen on swallowing. The subject complained : " that the neck of his shirt had been very tight for the last five or six days ; and that he could only button it by pulling the collar band up above the swelling ; that he slept a great deal ; that he was light-headed ; that there was much throbbing in the neck." The measurement was thirty-four and a quarter centimetres.

10.4.—No appreciable change was observed since the date of the previous note. The neck measured thirty-four and a half centimetres.

15.4.—The thyroid gland showed well-marked enlargement over its whole extent, but especially of the left lobe. The neck measured thirty-five centimetres. The subject was photographed. (Fig. 4.) He had been quite unable to button the neck of his shirt, which previously fitted well, and had tied the button-holes together with cord. (Fig. 5.)

21.4.—The neck measured thirty-five centimetres. There was no change since date of previous note.

1.5.—The neck measured thirty-four and a half centimetres.

9.5.—The neck measured thirty-four and a quarter centimetres, but looked decidedly smaller than on the 15th April. The subject was noticed to be somewhat anaemic. The experiment was discontinued and the subject placed under treatment by thymol and tonics. No trace of enlargement could be detected one month later.

3. A.M.—15.3.—The thyroid gland was normal. The neck measured thirty-four centimetres. The subject was photographed. (Fig. 6.)

31.3.—There was considerable enlargement of the gland in the region of the isthmus, which could be felt as a rounded boss under the finger, and was noticeable on swallowing. The neck measured thirty-four and a half centimetres.

6.4.—The right side of the neck was fuller than at the previous examination. The swelling of the isthmus was more marked, and, on digital examination was found to be dumb-bell-shaped. The left side of the dumb-bell was larger than the right. The swelling of the isthmus was well seen on swallowing, but it partially disappeared behind the sternum when the subject was at rest. The subject complained of tightness of his collar and throbbing in the neck. Measurement showed the neck to be thirty-five centimetres in circumference.

15.4.—The neck measured thirty-five and a half centimetres and the swelling of the thyroid and especially of the isthmus was more noticeable than at the last examination.

21.4.—There was no change to be found on physical examination. The subject was photographed. (Fig. 7.)

31.4.—The neck measured thirty-four and a half centimetres, and the thyroid appeared to be slightly smaller.

9.5.—There was no noticeable alteration since the day of the last examination. The neck measured thirty-four and a half centimetres. The experiment was discontinued and the subject placed under treatment.

4. A.D.—15.3.—The thyroid gland was normal. The circumferential measurement of the neck was thirty-four centimetres. The subject was photographed. (Fig. 8.)

31.3.—The neck measured thirty-five centimetres. There was some fullness under the sterno-mastoids, and the whole outline of the gland was readily seen when the subject swallowed. The subject did not complain of any symptoms.

6.4.—The neck measured thirty-five and a half centimetres. There was no appreciable difference from the appearances observed at the date of the last note.

15.4.—The neck measured thirty-five and a half centimetres. The subject complained of no symptoms though the gland was seen to be undoubtedly, but slightly, enlarged. The subject was photographed. (Fig. 9.)

9.5.—The neck measured thirty-five and a half centimetres. There was no further change to be recorded. The experiment was discontinued and the subject placed on treatment by thymol. No trace of enlargement could be found fifteen days later.

5. F.A.—In this case the original measurement of the neck was thirty-two and a half centimetres. On the twentieth day of the experiment the subject complained of a sense of fullness in the neck and of tightness of his collar band. The neck then measured thirty-three and a half centimetres. There was some fullness under the sterno-mastoids, and the outline of the gland was well marked and easily palpable. No further enlargement occurred, and on the 15th April the gland was found to have returned to its normal size.

EXPERIMENT J.—Carried out from 15th March to 9th April, 1910.

The conditions of this experiment were in all respects similar to those of Experiment I, with the exception that the *boiled* residue was given instead of the untreated residue. Ten individuals, of whom the average age was twenty-two, volunteered for the experiment. Amongst these were five in whom the thyroid gland was larger than normal; it was considered that these subjects might respond more readily to goitrous influences than those in whom the gland was perfectly normal.

The duration of the experiment was fifty-five days. It was carried out concurrently with the preceding one. In no case could the slightest increase in size be detected by any method of examination. In the five cases, in whom the thyroid was normal at the commencement of the experiment, there was no alteration whatever. In the other five subjects, in whom at the commencement the gland was somewhat larger than normal, the original and final measurements were as follows:—

	Original Measurement.	Final Measurement.
D. B.	34	$33\frac{1}{2}$
T. R.	34	33
D. R.	32	$30\frac{1}{2}$
K.	35	$34\frac{1}{2}$
R.	$33\frac{1}{2}$	33

These results show that the tendency to alteration in size of the thyroid was, in these five individuals, in the direction of diminution and not of increase.

The results of the foregoing experiments may be summarized as follows:—

1. Of twenty-three individuals who consumed the suspended matter of goitre-producing waters, six showed an increase in size of the gland, which persisted in a more or less well-marked manner up to the end of the experiment. Three others showed a thyroid hypertrophy of a transitory character.

2. Of twenty-three individuals who consumed the *boiled* suspended matter of goitre-producing waters none showed the slightest tendency to an increase in size of the thyroid gland.

These results are to be contrasted with those of my former series, which were:—

1. That of thirteen individuals who consumed the untreated residue of the goitre-producing waters of Kashrote, four developed a noticeable swelling of the thyroid gland, while two others showed an increase in size of the organ demonstrable by measurement and evident to the touch.

2. That of eight individuals who consumed the *boiled* residue of the goitre-producing waters of Kashrote, none developed any swelling of the thyroid gland, and this although three were individuals peculiarly likely to respond to goitrous influences.

The combined results of both series of experiments show:—

1. That of thirty-six individuals who consumed the untreated suspended matter of a notoriously goitre-producing water, ten developed a noticeable hypertrophy of the thyroid gland, while five showed a swelling of the organ of a transitory character.

2. That of thirty-one individuals who consumed the same suspended matter *which had been previously boiled*, none showed any reaction in the direction of increase in size of the thyroid gland.

The results of these experiments justify the following conclusions:—

1. There exists in suspension in the water of goitrous localities some unknown agent which is capable of initiating an hypertrophy of the thyroid gland.

2. This agent can be destroyed by boiling for ten minutes.

III. THE EFFECT OF THE ADMINISTRATION OF THE FILTERED GOITRE-PRODUCING WATERS TO MAN UNDER EXPERIMENTAL CONDITIONS

Having demonstrated that there exists in suspension in goitre-producing waters a substance which is capable of initiating an hypertrophy of the thyroid gland, and that this substance is readily destroyed by boiling, it became necessary to ascertain by carefully conducted experimental observations whether filtration deprived the water of its goitre-producing properties. For this purpose seven individuals were selected who consumed the filtrate of Kashrote water at the same time that the subjects of Experiment I were consuming the suspended matter separated from it by filtration. The same minute precautions were adopted in the case of these men as in those of Experiment I. In four of these the thyroid gland was perfectly normal on the 15th March, when the experiment commenced; the other three were the subjects of incipient goitre. All seven drank only the filtered Kashrote water for fifty-five days, with the following results:—The four normal individuals showed no change whatever, while the three men in whom the thyroid was enlarged showed a considerable reduction in size of this organ. This result indicates that the process of filtration renders water innocuous which was previously goitre-producing; the

suspended matter removed from the water by this process having actually produced a thyroid hypertrophy in five out of ten individuals who consumed it, while the subjects of the present experiment were consuming the filtrate. Water so purified not only does not cause a thyroid hypertrophy, but it exercises a curative influence on incipient cases of goitre.

The beneficial influence of filtration of goitre-producing waters is nowadays so well recognised, and has been demonstrated on such a large scale in the case of many public water supplies that it appears almost unnecessary to emphasize it. There are still, however, many scientific men who adhere to the chemical cause of goitre, and these are seeking in radio-active substances fresh support for their view. My experiments make it clear that, for the Hindu Kush region at least, where my researches have been carried out, the noxious principle of goitre is found only in suspension in the water and does not exist in solution; and, that if the cause of goitre is a chemical one it is of such a nature as to be unable to pass through pores of a Berkefeld filter and to be readily destroyed by boiling.

The hypertrophy of the thyroid gland, which is capable of being induced in the way described in the foregoing experiments, exhibits the following characteristics:—

1. It makes its appearance usually between the tenth and the fifteenth days of the experiment.

2. It shows a marked tendency to fluctuate in size.

3. It reaches its point of maximum size between the twenty-fifth and thirtieth days of the experiment.

4. It may completely disappear under the conditions of the experiment.

5. The hypertrophy of the gland is not great nor is it progressive under the conditions of the experiment. The tendency being towards a diminution in size from the thirtieth day onward.

6. It is accompanied, as a rule, with certain subjective symptoms of throbbing in the neck, feelings of fullness and discomfort.

The fluctuations of the thyroid gland observed in the individuals under the conditions of my experiments, exhibit a very striking resemblance to those of the endemic of goitre which are known to

occur in large communities. The endemic is subject to periods of increase and decrease. It shows a marked tendency to increase in an infected locality till a point of maximum intensity for that locality is reached, after which the disease tends to decline. This increase or decrease of the endemic in a given locality is rarely uniformly progressive but exhibits a marked periodicity.

Notes on Secondary Factors in the Production of Goitre

In the report of my first series of experiments on man, carried out on the lines which I have here indicated, and which was published in full in the 'Quarterly Journal of Medicine' (April, 1909), I made the following comments with reference to artificially produced thyroid hypertrophy:—'The conditions of these 'experiments differed from those under which the inhabitants of 'Gilgit live in certain important respects. The men were not 'subjected to the debilitating influences of defective hygiene, 'vitiated atmosphere, imperfect dietary, endemic disease and the 'like. Nor did that potent source of infection among an agricultural 'community, namely, contamination of the hands and food by the 'soil of an infected locality come into operation in their case. It is 'to the absence of such influences as these that I attribute the fact 'that the experimentally produced goitres were neither large nor 'progressive. . . . . I am convinced, therefore, that there are 'conditions provided by a residence in a goitrous locality apart from 'the water supply which are important determining factors in the 'production of the disease, and that in the absence of these 'secondary factors the organism, which is the real causal factor is 'of feeble pathogenicity.' My study of goitre during the past eight years has led me to attach great importance to the following factors as possessing a marked secondary influence on the development of thyroid hypertrophy in endemic localities:—

A. Factors directly influencing the thyroid gland which render it less able to counteract the action of the toxic agent of goitre without undergoing hypertrophy:—

(*a*) Hereditary influences: Other things being equal, the children of goitrous women appear to be more likely to develop goitre than the children of normal women.

(*b*) The marked influence of age, sex, puberty, menstruation, pregnancy, sexual activity on the functional activity of the thyroid gland: the added strain of goitrous influences at a time when the gland is already working at high pressure very markedly favours the development of a goitre.

(*c*) The influence of unhygienic conditions of life: defective air space, on which the so-called 'epidemics' of the disease are largely dependent; improper food or defective food supply; damp soil; the 'causes multiples' of the older French writers.

(*d*) The influences of certain infectious diseases on the thyroid gland such as rheumatism, rheumatoid arthritis, malaria, etc.

(*e*) The influence of emotional states.

B. Factors which favour infection:—

(*a*) The influence of occupation and habits of life, whereby individuals are rendered more liable to infection from the soil which is the natural habitat of the toxic agent of the disease.

(*b*) The influence of temperature: goitre is more likely to develop in temperate climates or at temperate seasons of the year.

(*c*) Susceptibility: new-comers to a district are very prone to contract the disease.

C. Factors favouring the action of the virus of the disease at the time of its entry into the body:—

(*a*) An organically impure water.

(*b*) Much mineral matter in suspension in the water.

(*c*) Very hard waters, especially those containing much lime, magnesium, or iron in solution.

These factors, I believe, act by inducing abnormal states of the lining membrane of the gut, and thus favour the development or action of the toxic agent of goitre. To them subsequent experience may add others, but they are those which I have found to be of the chief importance in the regions where my researches have been carried out. These influences are, in short, those which observers

from the remotest times have considered to be primarily causal in the production of the disease. Their importance is undoubtedly great, but secondary only to the true causal factor which remains still to be discovered.

IV. THE ACTION EXERCISED BY THE LACTIC FERMENTS WHEN APPLIED TO THE TREATMENT OF GOITRE

I have previously drawn attention to the curative action of intestinal antiseptics, notably thymol and beta-napthol, in cases of goitre, and I have regarded the action of these drugs as strong, though not conclusive evidence that the responsible agent in the production of the disease has its habitat in the intestinal tract of man. The action exercised by the lactic acid ferments when applied to the treatment of goitre affords additional evidence in favour of this view. In carrying out this line of treatment I have used the fresh cultures in milk of the *Bacillus bulgaricus*. I have treated up to the present time only eight cases in this way, but the results have been so striking that it is necessary to record them in this place. I have hitherto employed only milk as a medium for the administration of this bacillus but, owing to the scarcity of milk in this country, some other medium must in future be employed. Twelve to twenty ounces of 'soured milk' were given to each patient every morning before the first meal of the day for periods of one month to six weeks. The cases were all of several months standing, and during treatment there was no change whatever in the manner of life of the patients. They were treated as external cases, and carried out their work in the fields as usual. The results cannot be attributed, therefore, to change of locality, habits of life or water supply. Of these eight cases four were cured, two improved, and two showed no appreciable difference after six weeks. In those cases which were benefited by the treatment, it was observed that the thyroid gland began to show evidence of diminution in size about the tenth day of treatment, and, that the patients lost flesh. This latter fact is of considerable interest, as it is observed to occur also in the treatment of goitre by means of thymol, beta-naphthol and iodine. Figs 10, 11 and 12 represent various stages in the treatment of one of these cases. Fig. 11 shows the case after fifteen days, and Fig. 12 after thirty days of treatment.

V. THE EFFECT ON DOGS OF THE ADMINISTRATION OF EXTRACTS FROM THE FAECES OF GOITROUS INDIVIDUALS

EXPERIMENT K.—Carried out from the 10th December, 1909, to 8th March, 1910.

Nine healthy puppies were confined in a pen for a period of eighty-eight days and were fed during this time on watery extracts from the faeces of goitrous individuals. All due precautions were observed to prevent infection from other sources, such as the provision of a pure water supply, etc. Three control animals of a like age were confined in a neighbouring pen for the same length of time. The results were entirely negative. Post-mortem examination of the thyroid gland in these animals revealed no deviation from normal in the direction of hypertrophy. The thyroid gland of the nine puppies, to which extracts of the faeces were given, varied in weight from one-twelve-hundredth to one-twenty-four-hundredth part of the body weight. In the case of the control animals the weight of the thyroid varied from one-thousandth to one-fifteen-hundredth part of the body weight.

VI. RESULTS OF THE RESEARCH

1. There exists in suspension in waters which are known to be goitre-producing an agent which is capable of initiating an hypertrophy of the thyroid gland.

2. This agent is destroyed by boiling, and is removed from the water by filtration.

3. This agent is, therefore, either a living organism or a chemical substance the noxious properties of which are destroyed by heat.

4. The incubation period of experimentally produced goitre is usually about ten to fifteen days.

5. Goitre can be cured by the administration of intestinal antiseptics. The lactic ferments exercise a curative action when applied to the treatment of incipient goitres.

6. It is very probable that the agent which is responsible for the production of goitre is a living organism parasitic in the human intestine.

7. The disease cannot be communicated to dogs by means of watery extracts from the faeces of goitrous individuals.

These results confirm in detail those which I communicated to the Royal Society on 26th November, 1908.

EXPLANATION OF PLATES I AND II

Fig. 1.—'G.M.,' referred to in Experiment I. Shows appearance of neck at the time the experiment was commenced. Measurement, $33\frac{1}{2}$ centimetres.

Fig. 2.—The same subject. Photograph taken on thirtieth day of the experiment. Measurement, 35 centimetres.

Fig. 3.—'S.A.,' referred to in Experiment I. Shows appearance of neck at the time the experiment was commenced. Measurement, 33 centimetres.

Fig. 4.—The same subject. Photograph taken on the thirtieth day of the experiment. Measurement, 35 centimetres.

Fig. 5.—The same subject. Photograph taken on the thirtieth day of the experiment. Shows subject's method of fastening his shirt-band which buttoned comfortably prior to the commencement of the experiment.

Fig. 6.—'A.M.,' referred to in Experiment I. Shows appearance of the neck at the time the experiment was commenced. Measurement, 34 centimetres.

Fig. 7.—The same subject on the thirty-sixth day of the experiment. Measurement, $35\frac{1}{2}$ centimetres. The enlargement of the isthmus and of the right lobe of the gland is well seen.

Fig. 8.—'A.D.,' referred to in Experiment I. Shows the appearance of the subject's neck prior to the commencement of the experiment. Measurement, 34 centimetres.

Fig. 9.—The same subject on the thirtieth day of the experiment. The increase in size is slight but evident. Measurement, $35\frac{1}{2}$ centimetres.

Fig. 10.—Boy, aged twelve years, the subject of a goitre said to be of several months standing.

Fig. 11.—The same case after fifteen days' 'soured milk' treatment.

Fig. 12.—The same case after thirty days' 'soured milk' treatment.



1



2



3



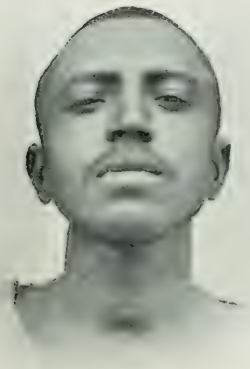
4



5



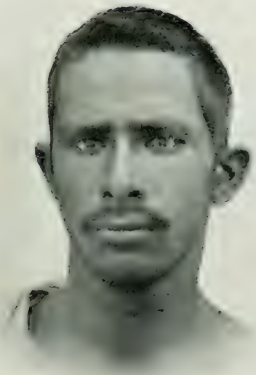
6



7



8



9



10



11



12

NON-ULCERATING ORIENTAL SORE: THE CULTURAL CHARACTERISTICS OF THE PARASITE AS COMPARED WITH A NEW SIMILAR PARASITE IN *ERTHESINA FULLO* (THUMB), A PENTATOMID BUG

BY

CAPTAIN R. MARKHAM CARTER, I.M.S.

(*Received for publication* 15 *February*, 1911)

In tropical and sub-tropical countries several peculiar types of skin lesion occur endemically and often limited in distribution to certain districts. These skin lesions have a very varied nomenclature but are loosely grouped under the name Oriental sore. In November, 1909, a brief differentiation between three types of Oriental sore was first made, and for the purposes of this communication, which is confined to experimental work on the parasites of the non-ulcerating type, as compared with a new and a similar parasite in *Erthesina fullo*, the three types of Oriental sore will be termed (1) the non-ulcerating Oriental sore, (2) the superficial flat Oriental ulcer, (3) the deep-seated Oriental boil. It is of interest to note that Thomson and Balfour* confirm the presence of the first type in Egypt, and wholly agree with me as to the possibility of there being different varieties of Leishmania hitherto undifferentiated.

Recent experience has shown that the non-ulcerating type of Oriental sore is in India and neighbouring countries a not uncommon affection. In a mixed population of over 2,000 patients from all parts of Northern, Western, Eastern and Central India, Burma and Assam, who sought anti-rabic treatment at the Pasteur Institute of India, seven cases of non-ulcerating Oriental sore were seen in

* Jour. R.A.M.C., Jan., 1910.

sixteen months. Neither of the two other types occurred, though all three were sought for. From the characteristic painless indolent nature of this cutaneous lesion, it can, however, readily be understood how easily it is overlooked unless seen in an advanced condition, or in a site such as the face or hand under frequent observation. It is thus probable other early cases in this number of patients escaped notice.

The history of these seven cases is as follows:—

Case I.—Boy, —— Singh, aged 19, native from Patiala State. Patient presented a large swelling on the right cheek, similating a gumboil. In the centre of this swelling was a slightly raised area, masuring $\frac{1}{2}$ inch by $\frac{3}{4}$ inch, pale straw in colour, and covered with fine papery scales. The edge of this raised area was slightly indurated and could be differentiated by the touch from the soft resilient raised patch.

The upper right eyelid presented a small circular raised nodule, reddish in colour, about $\frac{1}{4}$ inch in diameter. The left forearm presented four small raised nodules, one exactly like that on the eyelid, the other three resembling the area on the face in all details. There was the scar of an old ulcer on the back of the left forearm, said by patient to have resulted from an injury. No other person in his family shows or has ever shown similar cutaneous lesions.

History of the lesions.—The spot on the cheek first appeared fourteen months previously. It started as a minute itching red spot, which in a few weeks became anaesthetic to the touch and gradually extended to its present size. Six months later patient noted a similar spot appearing on the front of his right forearm. The other lesions on the forearm and eye developed subsequently.

Patient is in the habit of working in the open air, inspecting fields, etc., at his village. He often sleeps in the open during the day. He was shown specimens of ticks and biting flies to see if he could recognise them. He recognised Tabanids, Hippoboscidae and Haematapota as common biting flies of his district attacking animals. Patient does not remember being bitten by either ticks or flies, but states he has seen Tabanids bite persons as well as animals. The bite has been described to him as not being painful, but bleeding freely after a few seconds' smarting.

He described other flies in his district that bite, especially very small species in the neighbourhood of hill streams. These probably are Simulium and mosquitos.

Smears were taken from each spot, and also of peripheral blood from the right arm and ear. The blood clotted with unusual rapidity.

In all slides from the spots the mononuclear cells were found distended with Leishmania-like parasites. There were many free forms, those in the mononuclear cells were mainly oval and vacuolated. The small torpedo-form described in Delhi boil was comparatively rare.

In the films of peripheral blood, malarial parasites alone were seen.

Repeated punctures healed up invariably within a few days. The patient's spleen and liver were slightly enlarged.

Case II.—Miss C., European, Medical Missionary from Gujrat, aged 24. Presented a small raised area of pale straw colour on the forehead. This area was smooth and hairless over the greater part of its surface, the periphery was covered with fine paper-like scales of epithelium. The margin was sharply defined by a fine reddish indurated line of inflammation, which faded for about $\frac{1}{4}$ inch into the normal surrounding skin. The affected area measured $\frac{1}{2}$ inch by $\frac{5}{8}$ inch. The lesion was completely anaesthetic. The red margin was sensitive. Miss C. names the affection 'Monghyr Phora,' this being the name given to this lesion in her district.

The history of the case is as follows :—Miss C. first noted a small, itching, red pimple, like the bite of an insect, on her forehead thirteen months previously. This shortly became anaesthetic, but gradually increased. When the area was ½ inch in diameter it was painted with pure carbolic. This treatment did not arrest the progress of the lesion. A second minute pimple of the same type as the first appeared on the point of the elbow some months later. Patient states she has had no fever for eight years. She presents no enlargement of liver or spleen. She has, however, noticed that since the appearance of the spot on the forehead she has been feeling ill.

Examination of blood films from punctures of each spot showed the mononuclears distended with parasites as in the previous case. This blood clotted very rapidly. The peripheral blood showed no parasites of any kind.

Miss C., when asked if she recognised three or more types of Oriental sore, stated three were recognised in her district : (1) ' Monghyr Phora,' above described, a non-ulcerating form ; (2) a large superficial ulcer, common on the hands, wrists, ankles, and feet, whose floor was composed of exuberant granulations covered with pus and débris—this type is known locally as ' Chambal ' ; (3) a deep crater with raised sides, the former filled with and the latter undermined by foul pus and epithelial débris, this is known as ' Delhi boil.'

Popular belief is that the cause of the first type is the bite of the sand-fly or mosquito.

Case III.—Mr. E., European, aged 45, from Scinde. Patient presents multiple infection with non-ulcerating Oriental sore. The first appeared at Gumbat, outside Kohat, as a minute itching pimple on the forehead. This has slowly increased for fourteen months, is of pale straw colour, and raised above the surface of the adjacent skin, from which it is separated by a red thickened edge. The affected area is under ½ an inch in its greatest diameter.

A second spot appeared on the back of the forearm eight months ago, this is similar to the first, but encroaches on to an old healed scar, which seems to have limited its spread on that side. There are seventeen other scars on this forearm, patient states they are different from ulcerating ' Scinde sore.'

A third similar area was noted on the front of the left wrist. It was larger but similar to the previous two described, covered with fine papery scales, and completely anaesthetic. Patient first had a small ulcerating ' Scinde sore,' 2¼ years previously on the forehead, this healed with difficulty and left a small, smooth, white, depressed scar. Four similar ' Scinde sores ' developed later on the forehead, and fourteen sores on and around the right elbow joint. The scars were most distinct, many of them had a brown tinge round their edges. The raised lesions were examined for parasites and a condition found as in the previous cases. The blood clotted rapidly. There was but little enlargement of the spleen. Patient had a malarial history, but no parasites were found in the peripheral blood. He describes three forms of sore in Scinde : (1) the flat indolent and often multiple ulcer, known as ' Scinde sore,' which is the commonest met with ; (2) the deep-seated affection like a deep boil or carbuncle ; (3) the non-ulcerating form, comparatively rare. Patient asked, as he had now had both the first and third varieties, if he was liable to contract the second.

Patient states the sore on the forehead appeared at the beginning of the hot weather. He attributes these to the bite of either mosquitos or sand-flies, which are commoner than other pests outside Kohat. None of his servants or friends were suffering from the same disease.

Case IV.—Ephraim, son of W., native Christian from Gujrat, aged 7 years 2 months. Patient presents a small raised pale brown area the size of a three-penny bit, ¼ inch from the lower edge of the right nostril. The skin in the centre is smooth, and is seen under a lens to be devoid of fine hairs. The edges are covered with fine papery scales, the margin of the affected area is dark and slightly more indurated than the adjoining healthy tissue.

This spot first appeared seventeen months previously at the commencement of the hot weather, as a small itching point in the skin which gradually increased in size. Though anaesthetic at the time of examination, patient's parents state the child complains of intense itching in the affected area at intervals of many days. The child suffers from occasional fever every fifteen to twenty days. There were no malarial parasites in the blood. The liver and spleen were normal. On looking at the area with a high-power hand-lens, the appearance of the affected skin was as if it were distended with fine saccules of serous fluid throughout the whole thickness of the skin. By this serous sacculation the affected area was raised $\frac{1}{4}$ of an inch above the surrounding normal tissues. No enlarged glands were noted in the neck.

Films were frequently made from the spot, by puncturing the area in the centre, also in the indurated margin. The blood clotted rapidly. The wounds rapidly healed. There were myriads of free parasites and the mononuclear cells were distended as in the previous cases.

One other person in the house was affected with a similar lesion.

Case V.—Minnie, daughter of W., native Christian, Gujrat, aged 1 year 8 months. Patient presented what looked exactly like herpetic affection of the mucous surface of the upper lip. However, where the upper edge of the spot met the skin of the upper lip the characteristic slightly indurated red margin was seen. The child looked very ill. The patient's mother states the spot first started one year previously as a small itching spot, which gradually grew.

History of constant fever attacks.—Patient has looked ill for months. Spleen and liver normal. Blood taken from the affected area clots rapidly and presents a similar condition to Case IV. These two last cases have lived in the same house since the birth of Case V.

Case VI.—Abdul Rahman, a Pathan boy from Kohat, aged 10 years. Patient presented a straw-coloured, hairless, raised area on the tip of the nose, which had spread symmetrically on each side towards the nostrils. The edges of the affected area was covered with papery epithelial scales. The fine thickened margin of the affection seen in previous cases was well marked. The history of the case was confirmed by the political naib tahsildar, who brought the patient to the Institute The child's attendant was well acquainted with the progress of the affection as he was a friend of the patient's parents.

Fourteen months previously the sore commenced as a small itching khaki-coloured spot. It spread for some three weeks fairly rapidly, then remained practically in the same condition as when it first came under examination.

The nose was slightly enlarged, the affected area was anaesthetic. No enlarged glands were found. The spleen and liver were of normal size. There was no history of fever. None of the child's relations were infected.

On puncturing the area and making films, the same conditions as in previous cases were noted. The blood clotted rapidly. There were no parasites of any kind in the peripheral blood. Both the patient and his guardian state that three kinds of Oriental sore are recognised in the Kohat district. The first is called ' Spumai,' a form that never ulcerates, and is extremely common. The second a flat ulcer, known locally by the name ' Aurungzebe phora,' and by Englishmen as ' Frontier sore.' The third is a deep-seated boil, with a deep-pointed core, this is comparatively rare, and is called locally ' Naroo.' The native treatment for the non-ulcerating type is to apply a wafer of flour soaked in hot oil to the affected site and its immediate vicinity as hot as the patient can bear it. The native opinion around Kohat is that the lesion follows on the bite of the sand-fly, and appears most commonly at the commencement of the hot weather.

Case VII.—Major C., British officer serving on the Aden Boundary Commission. A small typical non-ulcerating Oriental sore developed on the front of the wrist and lasted for eight months, when it was excised and the area painted with pure carbolic acid. The sore first developed when the Commission encamped at Sanawi at the

foot of the Jehaf plateau in South Arabia. Two Sepoys, one other British officer and a native servant developed similar sores at this camping site. The water supply of the camp was obtained from two wells situated in a belt of tamarisk, where the camels were kept during the breeding season. Films made from these cases showed swarms of typical parasites. The blood clotted rapidly, and though repeatedly punctured there never was any tendency on the part of the sores to break down.

Case VIII.— Gunner K, 4th Battery, Mian Meer. Patient presents multiple infection with what seems to be the flat ulcerating type of Oriental sore. There are eight small flat ulcers on and around the left knee, sixteen similar sores on the left leg, front of left foot and round the left ankle joint. Seven small ulcers occur round right knee on right foot and leg, one larger flat ulcer above the right buttock. There are twenty-five old white scars on left leg and foot, twenty-two scars on right leg and foot. One small ulcer presented itself on the inner side of the left eyebrow. Patient has only been stationed at Mian Meer. There was no history of syphilis. The first ulcer appeared eleven months previously. Examinations of all the ulcers showed bacterial invasion alone. The peripheral blood showed benign tertian parasites but no other protozoa. The blood clotted slightly more rapidly than normal blood, but much less rapidly than in the previous seven cases. None of the patient's comrades are or have lately been similarly affected.

The point of interest in the first set of cases as compared with the last are:—

1. Non-ulcerating character.
2. Increased coagulability of blood.
3. High constant infection of mononuclear cells.
4. Rare infection of polynuclears.
5. Long history, and appearance of other similar lesions at long intervals.
6. Possible infection from another case in close daily contact.
7. Primary itching followed by anaesthesia.
8. Lesion presents constantly a central smooth surface, papery epithelial scales at periphery, margin indurated and visible to the naked eye.
9. Liver and spleen unaffected.
10. Possible exacerbations at irregular periods.
11. General malaise.
12. Occurs at all ages and in Europeans as well as natives.
13. First area affected usually on exposed surface.

The method of staining adopted for all smears and films from the tissues of the patient or culture tubes was as follows:—To 10 c.c. distilled water add 12 drops of ripened Giemsa stain, shake, and allow to act on films for twenty minutes. Then rinse each film with tap water and stain in a watery solution of eosin 1 in 50,000, until the film, which was dark purple, has changed to a rosy violet,

and the erythrocytes are seen under a low power to be of a rosy pink colour.

The films should be fixed in methyl alcohol for five minutes and blotted dry before the stain is applied.

The best method of making films from culture tubes is described in my paper, 'British Medical Journal,' Sept. 11, 1909, page 650. In a differential leucocyte count made from the affected areas as compared with the peripheral and normal blood, the result in all cases showing protozoal infection presented a marked increase in the polymorphonuclears. Difficulty was experienced in making evenly spread films, as the blood drawn from the lesions clotted in a few seconds. No pain is felt whilst boring into the lesion with a sterile glass pipette until the point has passed through the anaesthetic layer into the deeper tissues, about a quarter of an inch from the surface. As a rule mononuclear cells are found distended with oval parasites; polymorphonuclear cells rarely included any parasites. Many free and dividing forms were seen amongst the cells. These results are very similar to those seen by Thomson and Balfour in Egypt.

The following work on the developmental forms of the parasite of non-ulcerating Oriental sore is based on the material taken from a series of cases in India during the last eighteen months. Flagellated forms were first obtained in November, 1908.

In order to obtain development of the parasites of non-ulcerating Oriental sore the method advocated in September, 1909, is the most certain. To four units of clear non-activated human serum add four units of non-activated red blood cells and mix freely in a bulbed pipette with three units of a mixture of sterilised citrate 10 per cent. and salt 0·75 per cent. When the mixture is complete add four units of a similar citrate solution which has been heavily infected with organisms expressed from a puncture of the infected area. By passing a fine glass tube under the skin surface in all directions from one point of puncture, a free discharge of straw-coloured fluid and blood is easily obtained on pressure, and contamination is reduced to its minimum point. The fluid expressed must be collected and discharged into the citrate as soon as possible, as it clots rapidly. It is necessary to aerate the

contents of the culture each day by drawing air up the stem of the pipette and shaking the mixture in the bulb. The cultures should be kept at 22° C. Under such conditions flagellates appear in forty-eight hours, living symbiotically with masses of cocci and bacteria. These flagellated forms increase rapidly up to 120 hours, and then degeneration processes set in.

In seventy-two hours flagellated organisms of two types are frequently found singly and in pairs. One is of monadine form and stains blue, the other is oval or circular, and stains rosy pink. In ninety-six hours large clusters of rosy pink bodies are found, some flagellated, others not. Amongst such clusters a smaller number of blue staining monadine flagellates are usually seen.

The process of development in culture is as follows:—The minute parasites packed within the mononuclear cells liberate themselves, and rapidly multiply, dividing by simple fission. Each daughter cell grows to the size of the parent cell and divides into two or more grand-daughter parasites. After a series of such divisions, differentiation takes place as revealed by staining films from cultures, certain of the parasites now stain a fine rosy tint. These rose-staining parasites possess but little nucleus or chromatin, they slowly enlarge and a flagellum is extruded from the extra-nuclear centrosome. The other type of parasite develops into an oval pyriform body, which increases in size and divides by fission several times. A flagellum with three plications is extruded from the extra-nuclear centrosome, and the flagellated daughter cell breaks away from a tangle of pale rose-staining material to which these parasites are frequently found attached. The flagellum is usually the same length as the body of the monadine parasites. The zooglea mass of rose-staining material above mentioned seems to have been extruded by the parasites as a protective measure to enable them to fix themselves to one small area whilst development and division by fission takes place. It is no uncommon thing to find groups of many hundreds of parasites in all stages of development, thus differing from the parasite of Delhi boil worked at by Row in 1908 and 1909 in non-acidulated human serum. Surrounding such groups masses of bacilli and cocci are seen growing symbiotically. Groups of eight or ten blue monadine flagellates have been seen surrounded by mixed colonies

of germs living in perfect harmony, thus differentiating their specific nature from the allied parasite that gives rise to Kala Azar. Further differences, morphological and cultural, between the three parasites will be dealt with later.

A curious feature in cultures of non-ulcerating Oriental sore, first noted by me in October, 1909, is the constant occurrence of enormous clusters of what seem at first sight to be giant cocci. These bodies stain purple, and have often a reddish margin or film round them. They vary from forms the same size as an erythrocyte to smaller forms, altogether like cocci, diplococci, etc. This suspicious and interesting point has since been confirmed by Thomson and Balfour in their work on non-ulcerating Oriental sore in Egypt. In the description of material from an affected area on the neck, they note in addition to the parasites found free and in mononuclear cells groups of what seem to be large cocci, also a number of pale blue homogeneous structureless masses—a condition which is seen to occur also in cultures of the parasite from the intestinal tract of *Erthesina fullo*, to be described later. In material from affected areas on the thigh similar coccoidal bodies were found. These observers describe these blue coccoidal bodies as four to six times the size of the small cocci present. They stain feebly in their centres, often present unstained areas, occur in clumps or pairs, and may resemble huge gonococci.

In the light of recent experiments I am of the belief that the life-history of the parasite of non-ulcerating Oriental sore is as follows:—

The cockle-shaped form found in the tissues and mononuclear cells represent the form of the parasite which multiplies in the cells of the host by simple fission. In their earliest form they are seen as an exceedingly minute protoplasmal ring, containing a dot-like nucleus. Such forms are occasionally seen amongst the more maturely developed forms.

After a series of divisions by simple fission in cultures, what would seem to be sexual elements are formed, which stain differently. These seem to pair with interchange of elements. From this point the cycle becomes obscure, and light alone is thrown by observations on the life cycle of a similar parasite to be

subsequently described. If dot-like forms are released from the female cell, as recently seen in *Trypanosoma gambiense*, on examination of infected salivary glands in the intermediate host, the tsetse, these elemental forms might well be the minute bodies occasionally seen in infected mononuclears.

With a view to throwing light on these points the subsequent series of experiments were performed.

Before describing these, a few remarks on the insect host are necessary.

The order of the Rhynchota is divided into three main sub-orders: the Heteroptera, Homoptera, and Phytophthires.

The sub-order Heteroptera is divided into two series. The Gymnocerata having conspicuous antennae and the Crypotocera having their antennae more or less concealed.

The Pentatominae is one of the most extensive of the nineteen sub-families of the Gymnocerata, and includes the largest number of common species. *Erthesina fullo* and *Halys dentatus* are two speckled drab-coloured species commonly found in India, and supposed by competent authorities, such as Maxwell Lefroy to be predacious habitually or occasionally. This insect is widely distributed throughout India, Burma, Assam, Java, Japan, Formosa, Hainan, China and Ceylon. On hatching from the egg, there are four nymphal instars, lasting roughly about a month before the adult stage is reached.

Mature specimens of *Erthesina fullo* are common in the Himalayas throughout the year. Larvae and nymphs are most frequently seen in the spring. The mature insect is readily attracted by light at night and enters houses freely. The younger forms can only be taken on the bark of trees, and have not been noticed to frequent human habitations. In connection with Rhynchota of similar habits, it is as well to recall Donovan's observations in Madras, where he noted *Conorhinus rubrofasciatus* a member of the Acanthaspidinae is a common blood-sucking insect of local distribution found at night, whose nymphs frequent corners and crevices in houses. This insect, though not commensurate in its range with the occurrence of Kala Azar, is of extreme interest, as both nymphs and adults suck human blood if opportunity is afforded. Other Rhynchota with similar habits are

C. infestans, fed by Darwin on his blood, and *C. sanguisiga*, found in Arizona, in which latter case the site of puncture from which the insect has sucked human blood becomes painful, inflammation and even pus formation ensuing.

The point of this seeming discursion will be seen later. Forty-three adult specimens of *Erthesina fullo* were dissected, and the contents of their intestinal tract examined. In forty-one insects the crop contained large numbers of a crithidial organism freely motile. In one the infection was scanty, and one insect was found negative.

A series of nymphs and adult specimens of *Erthesina fullo* were dissected, and it was found that infection of the intestinal tract was common in all stages of the insect's life history. No eggs were examined. A few specimens of *Halys dentatus* and another species, as yet undetermined, were dissected and found negative, an interesting point showing the selective preference shown by the parasite for one species of insect. The further selective preference shown by the parasite of *Erthesina fullo* for human blood in culture as compared with blood of other animals is highly suggestive, and opens a large field for research work to those interested in discovering the definitive hosts that transmit the parasites of Kala Azar and the forms of Oriental sore to man.

Adult specimens of *Erthesina fullo* fed readily on my blood, citrated, when it was presented to them. A series of insects were fed thus twice a week through the winter and thrived on the diet.

Stained films prepared from the gut contents, showed all stages of the parasite, from the small cockle-shaped form simulating Leishmania to pyriform and flagellated bodies of two varieties as seen in non-ulcerating Oriental sore. The parasites were found living symbiotically with myriads of cocci and bacteria in the crop and intestine.

First series of culture experiments:—The crop of an infected insect, having been dissected out, was placed in a drop of citrate solution, 1 per cent. with 0·75 per cent. salt, and kept in a moist chamber for twenty-four hours. When the contents were examined the same conditions were noted as occurred in material from freshly dissected insects. There was, however, a marked increase in the number of the flagellate forms.

Ten drops of citrate solution were infected with the contents of the crop of two infected insects, and a series of cultures were made with bulbed pipettes as before.

A. A mixture of human blood citrate solution and infected citrate in equal units.

B. Ditto.

C. Human serum and infected citrate, equal units.

A similar series of culture tubes were made up, in which the blood from the rat, guinea-pig, rabbit, fowl and lizard (*Agama tuberculata*) was put up with equal units of citrate solution and infected citrate.

A similar series of serum cultures from these latter were also put up, and controls in each case made without infected citrate. The result of these experiments is rather striking, showing the selective preference of this parasite for human blood. In a culture of this parasite in citrated human blood the whole culture was found seething with active flagellates in twenty-four hours. This rapid increase was still more marked in forty-eight hours. Large groups of flagellates and parasites in all stages were common. After 192 hours a few live flagellating parasites were seen, most of them were motionless. In fresh preparations put up under a vaseline ringed cover-slip, the same process of multiplication was watched daily. The flagellated parasites were found to arrange themselves in large clusters around air bubbles with their flagella attached to the under surface of the bubble. Death, apparently due to want of air, was seen to occur in this confined area, usually in 168 hours.

The formation of pairs of monadine flagellate and circular or oval bodies was noted frequently, a condition checked by other workers in the laboratory.

In human serum the parasites vanished in forty-eight hours, a few cyst-like bodies with thick capsules alone were seen.

In blood and serum cultures from the common grey rat, no increase in the number of the parasites took place. They became motionless in four hours and had vanished in ninety-six hours. In blood and serum cultures from the white rat the parasites were found still motile after twenty-four hours, but were all motionless,

and the slide showed no increase in the parasites in forty-eight hours. In cultures made from the blood and serum of the guinea-pig, fowl and lizard there was no increase, a few parasites only were found dead and motionless after twenty-four hours. In lizard and fowl's blood and serum the parasites became motionless in four hours.

In the blood of the rabbit many single flagellates were seen actively motile up to ninety-six hours. There were no groups formed by multiplication of the parasites. After 120 hours the parasites became sluggish and finally motionless. Stained films showed no evidence of multiplication at any time.

Several interesting points were noted both in the fresh and in stained preparations from the culture tubes.

In cultures of the parasite in human blood it was frequently seen that at about the end of twenty-four hours pairs of parasites of different form were found constantly in apposition. The one a monadine flagellated body, the other a small parasite either boat-shaped, oval, or round, which was apposed to the monadine form in the neighbourhood of the nucleus in the case of the oval form, and often near the commencement of the flagellum in the case of the early boat shaped type. The character of the two forms, as shown in the plates, will be seen to be of the same nature as seen in the parasites of non-ulcerating Oriental sore during the stage of numerical increase in culture tubes. Stained preparations showed the monadine form stained bluish, the cyst-like body in connection with it rosy pink and with a flagellum often attached. In the case of the minute boat-shaped form this usually stained a bright blue in the early stage, and contains the same structural elements as the early form of Leishmania. Increase in the number of the parasites continued up to 144 hours in citrated cultures of human blood. After 196 hours but few motile flagellates were found, the majority of the parasites were motionless, and many showed commencing disintegration.

The monadine flagellate type had a characteristic movement. The anterior part of the parasite moved to and fro with the flagellum. The posterior one-third rarely is distorted. The flagellum would give five or six flicks then the whole parasite would vibrate, next the body of the parasite rapidly curves in **S** forms

for a few seconds, and becomes quiescent for a short period. The tip of the flagellum is applied by the parasite to the edge of the red cell frequently as if deriving nutriment from its contents.

The flagellated oval or round form of the parasite streams slowly along the field of the microscope propelled by the flagellum.

A detailed description of the parasite when stained takes up space, and is best elucidated by accurate drawings, the morphological details of the various stages of this parasite are shown in the accompanying plates, all drawn with the large Abbé camera lucida and Bernhard's drawing table.

A point of interest, and which may throw light on the rosy zooglea formation seen in culture groups of the parasites of Kala Azar and the varieties of Oriental sore, is the formation of what I propose to term 'anchor cords,' first noted in the monadine flagellate form of this parasite.

On putting up this parasite with citrated guinea-pig's blood, as above, and examining fresh-ringed cover-slip preparations after four hours a curious condition was seen for the first time. A group of four monadine flagellates were noticed to leave a certain refractile glass-like spot on the slide, and with the aid of their flagella explore the various channels that lay between adjacent groups of red corpuscles. It was noticed they invariably returned to the same spot after their excursions, often almost beyond the limit of the field of the microscope. When the light from the condenser was markedly reduced it was now noted that the posterior end of each parasite was connected by a fine thread to a small spot in a minute patch of sticky material adhering to the slide, which material was faintly ground-glass in appearance. These 'anchor cords' were extremely fine and highly elastic, and enabled the free flagellated parasite to wander far from the central spot, but yet return to its starting point from any angle with absolute certainty. There was no coiling up of these threads as the parasites returned to their common central point in the zooglea mass, they contracted and expanded with the requirements of the parasite, but seemed to have an equal length as regards the extreme limit of distance to which the parasites are capable of wandering. Such an apparatus can have only one or both objects in view: exploration for food material or the opposite sex.

It is probable this morphological detail occurs at this stage in the life-history of other allied flagellates and has hitherto escaped notice. These 'anchor cords' are extremely difficult to focus, but once located were shown to and detected by two other workers in the laboratory.

A series of adult *Erthesina fullo* were placed in a sterilised glass box and fed only on distilled water in a pledget of sterile wool for three winter months. The air in the box was changed once a week. This shows the vitality of the insect and its parasite. On dissection, the crop of each insect was found distended to about the size of a pea, with clear greenish fluid.

Examination of this fluid revealed numerous clumps of pyriform forms and free flagellates. Earlier forms were seen in large numbers.

A similar series of experiments with a series of bloods as before gave the same results.

On separating a series of insects fed only on water for three months, an insect was noted to defaecate a large quantity of dark, watery, greenish fluid. On examination of this fluid it was found to contain large numbers of parasites in all stages, the flagellate forms were, however, all motionless.

On making citrated blood cultures of the fluid with human, rabbit, fowl, guinea-pig, and white rat's blood, and preparing vaseline-ringed cover-slip preparations, it was noted the flagellates previously motionless and seemingly dead had all become freely active in four hours.

In the case of blood cultures from the rabbit, fowl, and guinea-pig with this material, the flagellates again were found motionless in twenty-four hours, and disintegrating. In the blood of the white rat flagellating parasites were seen after twenty-four hours, but dead in forty-eight hours.

In human blood, however, it is interesting to note there was an enormous increase in the numbers of the parasites by the end of forty-eight hours. Many pairs of monadine flagellates and cyst-like bodies were seen. Parasites in all stages of development were found increasing in numbers up to 144 hours; from this point they grew more sluggish in their movements and became motionless and degenerated.

From a primary culture of the parasite in human blood which showed flagellates after ninety-six hours, a sub-culture was made into fresh citrated human blood.

This sub-culture showed freely moving flagellates in 120 hours, none could be found after 168 hours.

That mature insects void the parasite in all its stages indicates the method of infection of others of the same species. It is not uncommon to find several of these insects huddled together in one spot. The fact that motionless and seemingly dead flagellar forms capable of revivifying in a suitable medium and multiplying in it are voided, shows that infection of other insects need not necessarily be confined to the infection by cyst forms, a commonly accepted belief amongst some workers at this branch of protozoology.

In addition to the stages in the life-history of the parasite mentioned above, we note that in the ileum, and below the crop, many cyst-like bodies occur with granular contents. These, if a phase in the life cycle of the parasite, and not another gut organism, would seem to be the stage previous to the liberation of the earliest form of the parasite.

To review the points of similarity between the parasite causing non-ulcerating Oriental sore and that infesting the intestinal tract of *Erthesina fullo*.

They both multiply and develop pyriform forms, monadine and circular or oval flagellated forms, in a culture of human blood acidulated with sodium citrate. Neither parasite will develop in an alkaline medium. They both live and develop symbiotically with masses of cocci and bacteria.

Bodies like giant cocci and bluish homogeneous bodies are found in cultures of both parasites.

Pairs of dissimilarly shaped and staining flagellated parasites are seen in both cases, whilst in the parasite of *Erthesina fullo* cyst-like bodies have been found suggestive of the formation of multiple early forms from a fertilised female.

The crithidial parasite of *Erthesina fullo*, when examined in the living condition, affords many interesting points likely to throw light on the life cycle of allied parasites. When a drop of human blood-culture fluid has been kept under a vaseline-ringed cover-slip for eighteen hours, and a few small bubbles of air

similarly enclosed amongst the medium, the fully formed monadine flagellates are found often to have arranged themselves in clusters around the edge of the air space, with the tips of their flagella pointing towards its centre. The parasites do not leave the bubble, but remain vigorously swaying to and fro, as if having found air they fear to leave it. In early fresh-infected cultures of human blood one of the most striking and frequent features is the linking up of a small motile boat-shaped body to the fully-formed crithidial monadine flagellate. The smaller body is found frequently apposed to the body of the former at the root of the flagellum. It may, however, be apposed opposite the nucleus or even on the flagellum itself. Once the small body has fixed itself to the larger parasite it seems to become securely stuck to the site, and accompanies the flagellate monadine in its peregrinations without being dislodged. Such pairs are commonest seen in the first period of rapid multiplication of the parasites.

The monadine flagellate presents, as a rule, pointed ends, the anterior flagellated end tapers finely, and in many cases passes a marked way up the flagellum. Occasionally the monadine form presents a globose posterior end, looking like a pouch filled with numerous brightly refractile granules.

The monadine form presents in the fresh condition an easily seen granular nucleus, a clear centrosome with indications of a surrounding vacuole. Small golden granules are commonly seen at either end of the body of the parasite, but usually occur in greatest number in the posterior portion.

When the monadine forms die, one often sees in fresh specimens a clear vacuole-like body in the centre of the parasite. Occasionally one sees evidence of a kind of prolongation of the flagellum into the body of the parasite, giving it a bladed screw-like appearance. Other parasites after death are globose in their centres, this globular area is clear as a rule, whilst collections of golden granules are seen at the posterior end and round a dark pigmented area, anterior to the nucleus, probably the site of the extra-nuclear centrosome.

In the case of the monadine form found in apposition with cyst-like bodies at the level of, or in the neighbourhood of the nucleus of the former, a detailed description can be given, as it is

based on a large series of observations by myself and other workers in the laboratory.

The smaller rounded cyst-like body may, or may not, present a flagellum arising from a clear staining spot, the extra-nuclear centrosome. The body of the parasite in favourable cases is seen to have (1) a fine, clearly defined, smooth capsule; (2) a comparatively large area of scattered granular material; (3) the nucleus a large dark spot is seen in one portion of the cell. Round this nucleus are often arranged a ring of fine dark dots.

A reniform ground-glass area, looking like a vacuole, is occasionally seen separated from the cuticle of the cell by a series of dark dots, seemingly equidistant from each other. These dots vary in number and in position, they may be found in a loose clump at one side of the cell.

The monadine form may or may not present a certain degree of globular enlargement at the point of contact between the two bodies. Where the small cell lies apposed at the level of the nucleus this globular enlargement has been most frequently seen. The nucleus of the monadine form has a ground-glass appearance, and its centre is usually situated at the junction of the anterior and middle thirds of the body of the parasite. The flagellum is usually about the same length as the body of the parasite, and presents two to four wide undulations. The root of the flagellum passes down the sinuous tapering anterior point of the parasite to end in the immediate vicinity of the extra-nuclear centrosome. When this latter is seen clearly, it is usually recognised as a brilliant clear oval spot, with a dark edge to it, the whole surrounded by a fine band of ground-glass-like material.

Between the extra-nuclear centrosome and the filamentous anterior extremity an area thickly sown with fine granules is seen. The posterior third of the parasite may show two definite dark refractive dots or a varying number of granules. Occasionally a cyst-like body, non-flagellated, showing a reniform vacuole and a large number of granules, as it were boiling within it, has been seen. Further experimental work on the later stages of the mature parasites is necessary, and these cyst bodies are recorded without comment.

EXPLANATION OF PLATES

PLATE III

I.—Shows pairs of flagellated organisms from a culture tube of non-ulcerating Oriental sore. These stain differently; the blue monadine form, the rosy pink oval form.

These organisms are in relation with a group of large coccoidal bodies, whose nuclei stain dark-bluish purple, and are often surrounded by a fine zone of cytoplasm, staining pink.

Film made after seventy-two hours' culture at 22° C.

II.—A cluster of parasites in all stages of development in a ninety-six hours' culture of non-ulcerating Oriental sore kept at 20° C.

The parasites are seen living symbiotically with bacteria and cocci. A large clump of parasites showing early differentiation into the two forms which stain differently and present flagella seen at the edge of the clump.

Two rosy-staining zooglea masses are shown.

III.—Pairs of bodies seen in a culture of non-ulcerating Oriental sore seventy-two hours old.

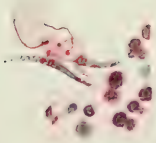
IV.—A group of rosy bodies, amongst which a few blue-staining monadine forms are seen. Note the group of coccoidal bodies, also the curious apposition of a flagellated rosy body to that of a blue monadine flagellate at the level of the nucleus.

From a culture of non-ulcerating Oriental sore ninety-six hours old.

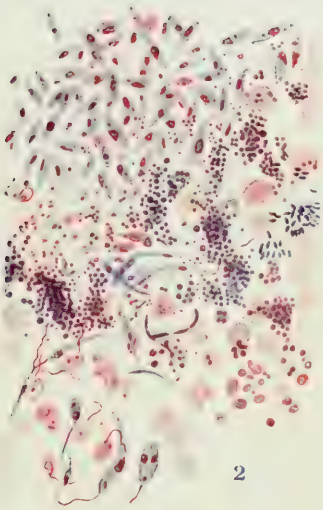
V.—Blue monadine flagellates, dividing forms, also others in conjunction with rosy bodies.

Note a globose enlargement in a blue monadine form opposite the point of apposition of a flagellated rosy body.

Drawn from a culture of non-ulcerating Oriental sore ninety-six hours old.



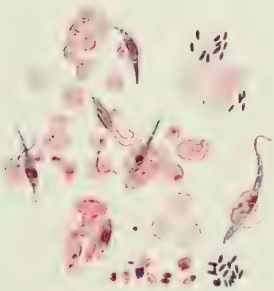
1



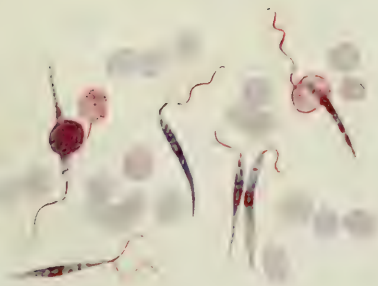
2



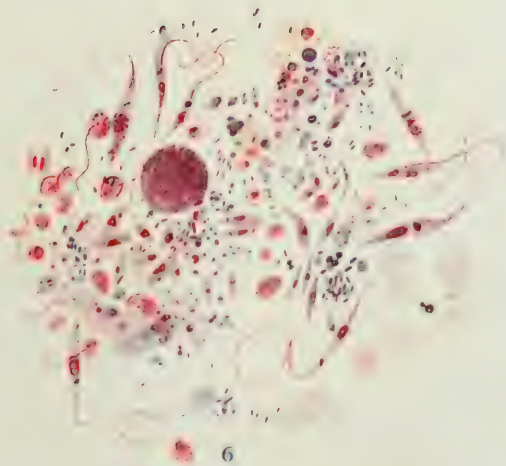
3



4



5



6

VI.—A group of organisms drawn from a culture of parasites from *Erthesina fullo* after seventy-two hours.

Note the parasites are seen in all stages of development, large blue coccoidal masses are seen attached to two rosy zooglea masses. The morphological details of the bluish monadine form are chiefly crithidial in type. The rosy bodies similate those found in non-ulcerating Oriental sore. The parasites are seen living symbiotically with bacteria.

Plate IV.

Series of drawings of living specimens of the gut parasite from *Erthesina fullo* in blood cultures. I-XII and No. XIX drawn with 1/12 objective No. 2 eye-piece, XIII-XVIII and No. XX drawn with 1/12 objective and No. 4 eye-piece.

I.—Oval body showing nucleus, nucleolus and vacuole.

II and III.—Monadine parasites (*b*) with small oval or boat-shaped bodies, (*a*) apposed at the root of the flagellum.

IV and V.—Flagellated large oval parasites.

VI.—Monadine flagellate (*b*), and small oval body (*a*). Note granular posterior and anterior ends of the former.

VII and VIII.—Flagellate parasite with the posterior ends globose and filled with granules.

IX and X.—A monadine flagellate dividing by fission from the anterior end of the parasite.

XI.—Monadine flagellate with globose granular centre.

XII.—Edge of an air bubble in a cover-glass preparation showing the arrangement of an aggregate of monadine flagellates, with their flagellae towards the centre of the lower surface of the bubble.

XIII.—A pair of parasites, the one (*b*) a monadine flagellate, the other (*a*) a cyst-like body unflagellated apposed at the level of the nucleus of the monadine parasite. The monadine parasite (*b*) presents a ground-glass-like nucleus about its centre. The posterior end of the parasite presents two dark dots. The anterior portion of the parasite presents fine granules and a small, clear, dark-edged oval area surrounded by a fine vacole. Occasionally the root of the flagellum can be traced ending in the neighbourhood of the vacuolar area.

The oval parasite (*a*) presents a fine capsule. To one side lies a large dark dot surrounded by a ring of fine dots. These adjoin a reniform ground-glass-like area, seemingly like a faint vacuole. This latter is separated from the capsule of the parasite by a line of equidistant dots of equal size.



XIV, XV, XVI and XVII.—Similar pairs of parasites, the oval parasite flagellated or not.

XVIII.—Cyst-like bodies packed with small golden granules, which seemed to boil within the capsule.

XIX.—Four monadine flagellates with acicular posterior extremities, each parasite attached by a fine elastic cord to a spot in a mass of zooglea material.

XX.—A parasite showing the posterior end of the parasite contains clear fine granules.

A and B show two positions of the parasite leaving the zooglea clear mass with the flagellated anterior extremity forwards.

b.—The fine elastic posterior 'anchor cord.'

INFANTILE LEISHMANIASIS (MARDA TAL BICCIA) IN MALTA

BY

A. CRITIEN, M.D., D.P.H., D.T.M. (Liv.)

(*Received for publication* 23 *January*, 1911)

There exists in these Islands a morbid condition characterised by great enlargement of the spleen and profound anaemia. It is met with almost exclusively in very young children, and is nearly always fatal.

Although, with the exception of splenic leukaemia, all the anaemias with chronic swelling of the spleen are clinically little differentiated, there always has been a feeling among local practitioners that they were dealing with a special pathological entity, which being in its clinical manifestations very much like some of the better known disorders of the blood and haematopoietic organs, they could not very well, in the absence of some special element of diagnosis, dissociate from the latter.

Etiologically its connection with syphilis, tubercle, rickets, or amyloid degeneration is not apparent; as to malaria, it is not endemic in these Islands. The disease is variously certified at death as leucocythaemia, splenic leukaemia, splenic anaemia, pseudo-leukaemia, splenitis, splenopathy; but in the Maltese language it is referred to by the professional and the layman alike under one name, ' Marda tal biccia.'

The disease begins very insidiously with spells of fever of a slow type, at a period of the child's life when slight ailments are very frequent and not made much of. If the initial pyrexia tends to establish itself without any obvious explanation such as dentition or gastro-intestinal troubles, Mediterranean fever is apt to be suspected, especially as on percussion the spleen is already found somewhat enlarged. More often, the initial attacks of fever do not attract attention until there arrives a time when the child, having lost its

usual brightness and desire for food, becomes pale and begins to lose flesh. By this time the spleen can be felt as a distinct tumour in the left hypochondrium, and the little patient is shown to a doctor. From this stage the malady has a protracted course of from six to eighteen or twenty months.

The following is a short clinical picture of the disease when fully developed:—The skin is waxy white or sallow, according as the subject is fair or dark; the lips and mucous membranes are blanched; the eyes are full of sadness and look abnormally large in the emaciated little face; all the muscles are flabby and more or less atrophic, the distended abdomen contrasting with the wasted thorax, its fulness more pronounced on the left. The outlines of the splenic tumour may sometimes be easily made out by inspection. On examination the spleen is found generally to extend down to the level of the umbilicus, firm but not hard, freely movable, only slightly tender on pressure or not at all, its margins rounded but well defined, its notches well pronounced. In growing downwards as a rule it keeps to the left of the navel, reaching very often down to the iliac crest; but in some cases it fills the pelvis, and, crossing the *linea alba*, occupies the right inferior quadrant, where in an extreme case I have found it in close apposition to the anterior border of the enlarged liver. The occurrence of the *caput medusae* is very common. The liver is also enlarged, but to a less degree, not more than one or two fingers' breadth below the costal margin. In the cases observed the presence of fluid in the abdominal cavity could not be detected. The lymphatic glands accessible to examination are not sensibly enlarged, but when the wasting is very pronounced they can easily be felt and seen. Transitory oedema of the feet, hands and eyelids is common. The appetite is very indifferent, but sometimes there is a great craving for food; it is rarely perverted: in some cases the patients pick and chew bits of plaster or little stones. Gastro-intestinal troubles are the rule, manifested by intercurrent attacks of very fetid diarrhoea. A symptom met with sooner or later consists of a solitary or repeated attack of dysenteriform diarrhoea with tenesmi, slimy motions containing blood and mucus or mucus only. The frequent passage of loose stools is not infrequently accompanied by temporary shrinking of the spleen. In one case this was observed to such a

degree and the improvement of the other symptoms was so marked and continued that the mother firmly believed the abdominal tumour—the spleen—had been passed with the motions. Curiously enough the case ended in complete recovery; the patient, a boy of six, when seen by me was in perfect health. Evidently, he also had had cancrum oris, as the upper middle incisors were missing and the gums were badly scarred. Intercurrent attacks of bronchitis are by no means rare. Epistaxis is a common occurrence, so is bleeding of the gums, and the appearance of one or more crops of purpura all over the trunk and face. In one case a few vibices were observed. A frequent terminal complication is cancrum oris. The mortification may assume formidable proportions in a few days, or it may evolve less acutely, death supervening in a month or six weeks. It sets in very stealthily, almost without pain, the increased flow of saliva at first being ascribed to irritation of the mouth due to dental evolution. The process in the cases observed started from the gums in connection with the upper incisors, lower or upper premolars. The gangrene is often very extensive and exceedingly repulsive to the eye and nose, and the deformity is generally such that no plastic operation could ever remedy. The blood in advanced cases is quite watery; it separates quickly into clot and plasma. Prognosis is very bad. Nearly all practitioners, however, quote from experience one or two instances of the disease ending in recovery; but some maintain that all recoveries are cases of mistaken diagnosis. It is wonderful how some patients can go on living; on the other hand death occurs when least expected. Bronchial complications are very frequent towards the end.

The disease, as outlined, was found to have reached a more or less advanced stage in the twenty-one cases, to which the following notes refer:—

1. Girl, 4 years, seen in May, 1909. Ill since October, 1908, after whooping cough. Intercurrent waves of fever of a remittent type, profuse perspiration, no appetite, anaemia, muscular atrophy, transitory oedema of both legs, spleen reaches down to iliac crest, liver also enlarged, purpura, dysenteric diarrhoea with great loss of blood, bleeding from gums, noma, fall of upper incisors. Peripheral blood examined: two Leishman-Donovan bodies in a large mononuclear, well-marked large mononuclear increase. Died about one month after. No post mortem or puncture of spleen after death allowed.

2. Girl, 4½ years, seen in July, 1909. Very scanty notes taken at the time. Died in January, 1910, after an illness of about fourteen months. Two weeks before death a swelling of the left cheek and a very foul condition of the mouth foreboded

the very common final complication, noma. The disease had started with spells of fever with very high temperature of a remittent type, then anaemia, great pallor of the integuments, enlargement of the spleen, intermittent attacks of diarrhoea, oedema of the extremities followed. Liver moderately enlarged, lymphatic glands not affected. No other cases in the same family. No dogs kept. Smears and sections from spleen post mortem : smears were literally studded with Leishman-Donovan bodies, but the parasites were not so abundant in the sections.

3. Girl, 3½ years, seen in October, 1909. Had measles in April, 1908. About four months ago had fever for twenty days, no high temperatures, no regular type. She gradually became anaemic and lost flesh. Now her skin is of an earthy pallor, marked muscular atrophy, no oedema, no haemorrhages, the appetite very poor, is at times abnormal, has diarrhoea every now and then, no blood with stools. Spleen is enlarged down to two fingers' breadth below the navel. Anterior border of liver is two fingers' breadth below costal border on mammillary line Glands not enlarged. No noma. Died about three months after.

4. Boy, 25 months, seen in October, 1909. Ill since six months. Mother did not notice any fever at first; two months after he had a spell of dysenteric diarrhoea with loss of blood. Now he is very pale and has lost flesh, very fretful, glands not enlarged, no oedema, fever of an irregular type. Spleen reaches down to three fingers' breadth below navel, no marked increase of liver. Mother stated that she had had the same disease when a child. Case has not been seen again.

5. Boy, 6 years, born in Malta of English parents, seen in October, 1909. Ill since one year. Spleen began to increase in size about five months ago. Extreme anaemia and wasting, spleen enormous, spells of fever every now and then, appetite good, no purpura, no bleeding from gums, no blood with stools, one or two vibices on the back. Later on had several purpuric eruptions, bleeding from gums, profuse diarrhoea. The doctor attending noticed an almost complete retraction of the spleen a few days before the end and the diarrhoea ceased, but there was no amelioration of the other symptoms. The child died of exhaustion in April, 1910.

6. Girl, 3 years, seen in November, 1909. Ill since one year. Moderate anaemia, no marked wasting, no oedema, has a temperature every now and then, appetite good. Spleen reaches to just below navel. Has had purpura and dysenteric attacks but no bronchial phenomena. Glands not enlarged. Blood smears from ear : no parasites. Child lost sight of. Doctor attending stated to have observed a great improvement following a course of injections of methyl arsenate of iron.

7. Girl, 6 years, seen in December, 1909. Ill since one year, after sustaining a fractured clavicle. Earthy colour, extremely anaemic; very extensive gangrene of gums, both lips, nose and cheeks. Great emaciation. Spleen, but for the enlarged liver which reaches to about four fingers' breadth below costal margin, occupies the whole abdomen; has dysenteric diarrhoea; appetite fairly good. Died four days after. No examination of the blood or spleen puncture allowed. A sister died from the same disease when two years old : had noma followed by same extensive gangrene. An elder brother, who is stated also to have had the disease when six years old, is now quite well. An aunt and a cousin on the mother's side are supposed to have died of the same complaint.

8. Boy, 3 years, seen in January, 1910. Ill since eighteen months, spells of fever with profuse perspiration, intercurrent attacks of diarrhoea and bronchitis, purpura, epistaxis, bleeding from gums, extreme pallor and emaciation. Face has a very old and sad look. The spleen free, firm, notchy, easily movable, not painful, reaches down to the inguinal fold; liver has grown to four fingers' breadth below the costal margin. Died suddenly in February. Two dogs had been in the house for a long time. A fragment of spleen obtained post mortem : Leishman-Donovan bodies in smears and sections.

9. Girl, 5 years, seen in January, 1910. Ill since November, 1909. Had whooping cough a year ago. The disease began with spells of fever and anaemia, then swelling of the spleen; this organ now reaches to inguinal fold; liver is little enlarged. She is very pale and very sad, has diarrhoea, no blood with stools, no epistaxis, no bleeding from gums, no purpura, feet are oedematous. A cousin on mother's side died from the same complaint when eighteen months old. Seen again in April, no change; administration of *Tr. senegae* suggested. Seen again in October: very marked improvement, the spleen has receded to one finger's breadth below the costal margin, the child has recovered her gaiety, has a healthy colour and good appetite. One dog in house.

10. Boy, $3\frac{1}{2}$ years, seen in January, 1910. Ill since seven months, after a fright, as stated. Spells of fever, loss of appetite, great pallor and emaciation, oedema of feet, hands and eyelids, great sadness, dysenteric diarrhoea and bronchial catarrh. The splenic tumour fills the left inferior and part of the right inferior quadrant; the liver reaches down to a finger's breadth below costal margin. Seen again in February, the spleen maintains the same curved configuration but does not reach quite down to the ilium, the liver is also smaller, the diarrhoea persists, general condition worse. Peripheral blood examined, no parasites. Died in November, 1910. A brother died of the same disease in June, 1907, when two years old, after an illness of fourteen months. No dogs.

11. Boy, 3 years. He is one of six, of which the eldest is 14 years old and the youngest 15 months. No other children have had the disease. Ill since fifteen months. It was only after three months of irregular fever that the enlarged spleen began to attract attention. Iron preparations were prescribed. After three months' treatment the splenic tumour was so reduced in size that the mother believed him cured. He then contracted whooping-cough and the spleen started growing again. About the time this patient sickened two other children living near were suffering from splenic anaemia, both developed cancrum oris and died. A dog was owned by these people. I saw the child in March, 1910, three days before death: great pallor and emaciation, oedema of feet and eyelids, spleen reaches down to one finger's breadth from iliac crest, liver moderately enlarged, diarrhoea, a black stool occasionally (melaena?). Cancrum oris started opposite right upper premolars, now mortification of right cheek, exposure of buccal cavity; no epistaxis, no purpura. Post mortem: Cancrum oris, extensive destruction of right cheek and gums, loss of teeth. Lungs: right, caseous lobular pneumonia; left, emphysema. Heart: all cavities dilated. Liver: enlarged, consistency increased. Spleen: weight $8\frac{1}{2}$ ounces, about three times normal size, rounded margins, many notches, very firm, capsule thickened and adherent; the cut surface greyish towards the middle, brownish-red at periphery, malpighian corpuscles prominent. Mesenteric glands enlarged, not caseous, the other glands normal in size and appearance, bone marrow body of femur reddish and swollen. Smears and sections of spleen and liver show a fair number of Leishman Donovan bodies. Smears from mesenteric glands, a few parasites present. Smears from bone marrow, owing to defective fixation, could not be stained successfully.

12. Girl, 4 years, seen in March, 1910. Has had fever and diarrhoea since three months, moderate emaciation, skin and mucous membranes anaemic, loss of appetite, the child is listless and sad. Diarrhoea every now and then, with tenesmi and passage of mucus, but no blood. Splenic tumour rather narrow, it does not reach below navel, easily movable, not painful; liver cannot be felt on palpation, lymphatic glands normal in size. Seen again in December. Abdomen greatly distended as the splenic tumour has grown down to the iliac crest and, to the right, under the linea alba, the liver is two and a half fingers' breadth below the costal margin, cervical glands are larger than normal, eyelids are oedematous, respiration is much hindered, hollow cough, fever, no haemorrhages.

December 3rd, 1910.—Peripheral blood examined: no parasites. Relative leucocytic values: Large mononuclear, 36·2; small mononuclear, 29·4; transitional, 7·0; polynuclear, 26·2; eosinophile, 1·2.

13. Girl, 3 years. One of a large family, but no other member ever had the disease. A child next door died of splenic anaemia in 1902. Patient has been ill since December, 1909. In January, 1910, had a slight attack of diarrhoea with tenesmi; after three or four weeks of fever, attended with profuse perspiration, mother noticed the splenic tumour just below costal margin. Seen by me in March: no marked emaciation, moderate anaemia, no oedema, no purpura, appetite fair, no great depression. Splenic tumour reaches down to three fingers' breadth below costal margin, liver is just palpable, no diarrhoea. Splenic puncture with an ordinary hypodermic needle, usual antiseptic precautions: Leishman-Donovan bodies present in a fairly large number. Treatment with senega preparations suggested. Marked improvement during the next two months; the case, however, ended fatally in September.

14. Boy, 21 months. Ill since August, 1909. Two cousins on mother's side of about the same age died from same disease after a year's illness. In August, 1909, after a fright, the boy started having a temperature at irregular intervals, with perspiration; several attacks of dysenteric diarrhoea, slight epistaxis and crops of purpura followed. Seen in March, 1910: great pallor of skin and mucous membranes, loss of flesh, profuse salivation, initial mortification of gums at the base of left premolars and slight bleeding, oedema of feet and eyelids. Spleen enlarged down to iliac crest, moderately hard, freely movable, not painful, very marked notches, liver just palpable. Died in April.

15. Boy, 19 months, seen in March, 1910. No history of splenic anaemia in the family—a large one. Ill since $4\frac{1}{2}$ months; spells of fever with perspiration. No diarrhoea, no bronchial catarrh, no epistaxis. Appetite good but perverted, is always picking and chewing stones. On examination: moderate anaemia, no great loss of flesh, gums normal, splenic tumour reaches down to iliac crest and to the right, $1\frac{1}{2}$ inches beyond linea alba, liver can be felt two fingers' breadth below costal border. Splenic puncture: Leishman-Donovan bodies present in all smears in moderate numbers. Treatment: tinctura senegae in large doses. Seen again in April: extreme pallor, slight bleeding from gums, purpura. Died the same month.

16. Boy, 18 months, seen in April, 1910. There is a history of short spells of fever before mother noticed that spleen was enlarged, five months ago. Since then has had diarrhoea off and on, slight bleeding from gums, a few spots of purpura, no oedema. Now skin and mucous membranes anaemic, loss of flesh, appetite fairly good, spleen reaches down to iliac crest and laterally almost to umbilicus, caput medusae, liver one finger's breadth below costal margin, lymphatic glands not enlarged. Splenic puncture: no parasites met with. Died in August.

17. Boy, 2 years. Weakly child from birth. Seen in April. About two months before had enteritis with tenesmi, passage of mucus but no blood, no haemorrhages. Cancrum oris started three weeks before I saw him, when a small slough formed in the gums over the upper incisors. Mother never noticed any enlargement of spleen. Now great pallor and emaciation, diarrhoea, prolapsus ani, mortification of upper lips, nose, cheeks and lower eyelids. Spleen and liver can hardly be felt on palpation, but abdomen is very distended. Died 20th April, 1910. Post mortem, partial: spleen exceeds costal border by about two fingers' breadth. Spleen smears swarming with Leishman-Donovan bodies. About a year ago they had a small dog in the house. Eldest sister died five years ago; two other boys and a baby, of whom the former are older than patient, all alive. Eldest sister was ill for one year, and presented the following symptoms: progressive anaemia and emaciation, fever, attacks of diarrhoea with passage of mucus and prolapsus ani, but no enlargement of spleen was noticed by mother. Then gums in connection with lower left molars underwent a process of mortification which extended to cheek, sloughing through. At that time they also kept a dog different from one mentioned above.

18. Girl, 2½ years. Seen in May, 1910. Ill since five months: Anaemia, emaciation, loss of appetite, diarrhoea with passage of mucus and tenesmi. Now great dejection, bronchitis, no oedema, splenic tumour reaches to about four fingers' breadth below costal margin, liver enlarged but to a less extent, lymphatic glands normal. Died August, 1910.

19. Boy, 15 months. Not seen during life. Post mortem, twenty-four hours after death, 2nd June, 1910: extreme anaemia, mucous membranes bleached, no great emaciation, spots of purpura, oedema of lower limbs, liver very large especially left lobe, splenic tumour reaches to about two fingers' breadth from iliac crest, and is pushed to the left by the enlarged liver, cancrum oris with loss of upper incisors, mortification of gums, upper lip, left cheek, and nose up to lower eyelids on both sides, lymphatic glands normal. Spleen: 5 inches by 3 inches, weight 6 ounces, perisplenitis, patches of infarction, free edges rounded, deep notches, firm but not hard. Liver: 7 inches by 4½ inches, weight 14¼ ounces, uniform yellowish white colour, on section very anaemic, dry, mottled appearance. Abdominal cavity contains a small amount of clear yellowish fluid. Mesenteric glands colourless, normal size. Lungs very anaemic, otherwise normal. Heart flaccid and anaemic, pericardium contains a moderate quantity of clear transparent fluid. Smears and sections from spleen, liver and kidneys contain Leishman-Donovan bodies, numerous in the spleen, very few in the kidneys.

20. Girl, 2 years. Ill since April, 1910. At first fever of slow type lasting two weeks. In September the child, who had acquired a sickly hue and lost flesh, started again having a temperature, and by the middle of November the spleen was so enlarged as to be easily palpable. Had no diarrhoea, appetite maintained, no petechiae, no epistaxis, slight bleeding from gums, no oedema. She is the fifth child in a family of six, all in good health; but a cousin on the mother's side died of the disease. No dogs kept. Seen on the 25th of November: very pale and emaciated, no oedema, no purpura, has a hollow cough. Spleen comes down to the level of navel, liver is not palpable. Abscess the size of a large walnut just behind angle of right mandible, gums normal, diarrhoea with tenesmi and passage of blood-tinged mucus. Spleen puncture: Leishman-Donovan bodies in large amount, mostly free, some in large mononuclears, others in groups of 8 to 11.

21. Girl, 2½ years. Ill since three months (?). Seen in December, 1910. Great anaemia and emaciation, rise of temperature at irregular intervals, diarrhoea with tenesmi but no blood, cancrum oris starting over first left upper premolar, cheeks swollen hard and tender, has had no haemorrhages, no oedema, accessible lymphatic glands normal, appetite maintained. Spleen freely movable and painless, occupies upper and lower left quadrant, the liver reaches down two and a half fingers' breadth below costal margin. P. is the youngest of five, of which two died when quite small, eldest two are living and in good health. A dog kept up to ten months ago. Splenic punctures: Leishman-Donovan bodies present in fairly large numbers.

For the observation of these cases I am indebted to the kindness of medical colleagues practising in different parts of the Island, especially Dr. Cannataci, Dr. Wirth, and the staff of the Central Hospital. The symptomatology, the age of the patients, the almost constantly fatal termination, all point to a common morbid condition. It is likewise justifiable to infer that the majority of deaths catalogued under the different names referred to before, more especially if belonging to a certain age period, are instances of one and the same disease.

The question now arises whether all the deaths due to this disease are caused by *Leishmania* infection. Up to the time of writing* *Leishmania infantum* has been found in nine out of ten cases by examination of the spleen and other organs, and in one out of three cases in which films of peripheral blood were stained. These facts do not as yet justify generalisation, the more so as no case of infantile leishmaniasis should be counted as such unless the clinical diagnosis be supported by the demonstration of the specific protozoa; but they go very far to show that the bulk of deaths under five certified as due to leucocythaemia, splenic leukaemia, pseudo-leukaemia and splenitis are cases of *anaemia infantum a leishmania* (G. Pianese, 1905), or *infantile kala-azar* (C. Nicolle, 1908).

The conditions I have found associated with the disease will now be reviewed. The disease is not notifiable, hence all considerations regarding its incidence are based on the deaths imputable to it. Having regard to its fatality, the deaths must fall very little short of the number of attacks.

Locality. During the ten years, 1899-1908, 686 deaths under five were registered in Malta and 58 in Gozo. In order to form an idea of the prevalence of the disease in the various localities the population under five for Malta and Gozo and for the different populated centres in Malta, as estimated in the last census, 1901, have been divided into the number of deaths for the said decennium and the results multiplied by 100. The figures obtained may be taken to represent a sort of endemic index expressing with some degree of approximation the intensity of the disease in the different

---

* The following are some notes kindly given me by Captain W. L. Baker, R.A.M.C., of a case under his care :—

Male child, aged 19 months. First seen end of June, 1910. Spleen then enlarged to umbilicus.

Blood Count ... R.C. 4,000,000 per c.cm.
W.C. 4,400 ,,
Hgb. Index 25 per cent.

Spleen became more enlarged and child developed diarrhoea with passage of blood, small haemorrhages also occurred in skin of limbs and trunk. Anaemia increased, and in the middle of August the count was :—

R.C. 2,050,000 per c.cm.
W.C. 3,000 ,,
Hgb. Index 20 per cent.

Before death, which occurred early in September, spleen retracted two fingers' breadth above umbilicus. *Leishmania* sp. found post-mortem in spleen in enormous numbers, and in lesser numbers in liver.

places. Populations under five, deaths and indices have been tabulated as follows:—

Table 1

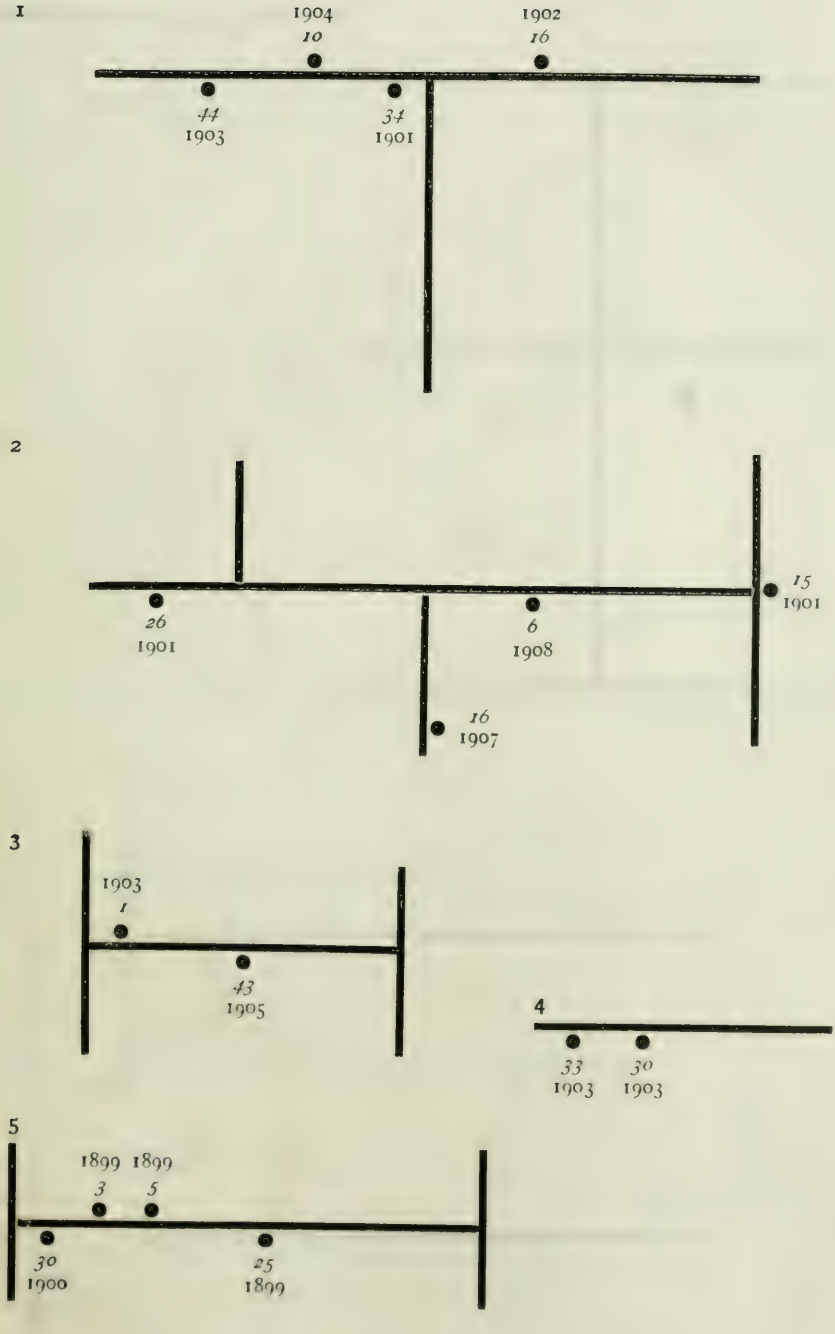
Locality	Persons under 5. Census 1901	Deaths, 1899-1908	Endemic index
Malta	19,684	686	3·4
Gozo	2,256	58	2·5
Malta—			
Valletta	2,295	40	1·7
Floriana	559	4	0·7
Senglea	914	10	1·0
Cospicua	1,475	43	2·9
Vittoriosa	693	14	2·0
Calcara	128	17	13·2
Zabbas	720	24	3·3
Tarxien and Paola	576	40	6·9
Zeitun and Marsascirocco	908	55	6·0
Asciak	217	20	9·2
Luca	335	13	3·8
Gudia	117	6	5·1
Chircop	82	9	10·9
Micabiba	158	12	7·5
Safi	53	4	7·5
Zurrico	452	38	8·4
Crendi	192	17	8·8
Misida and Pieta	539	21	3·8
Sliema and St. Julians	1,415	39	2·7
Hamrun	1,394	40	2·7
Birchircara	1,057	49	4·6
Curmi	1,193	22	1·8
Zebbug	624	20	3·2
Siggieui	395	32	8·1
Balzan Lia and Attard	464	16	3·4
Naxaro	414	6	1·4
Gargur	170	1	0·5
Musta	635	29	4·5
Imgiar and St. Paul's Bay	117	5	4·2
Melleha	372	2	0·5
Rabato and Dingli	1,007	36	3·5

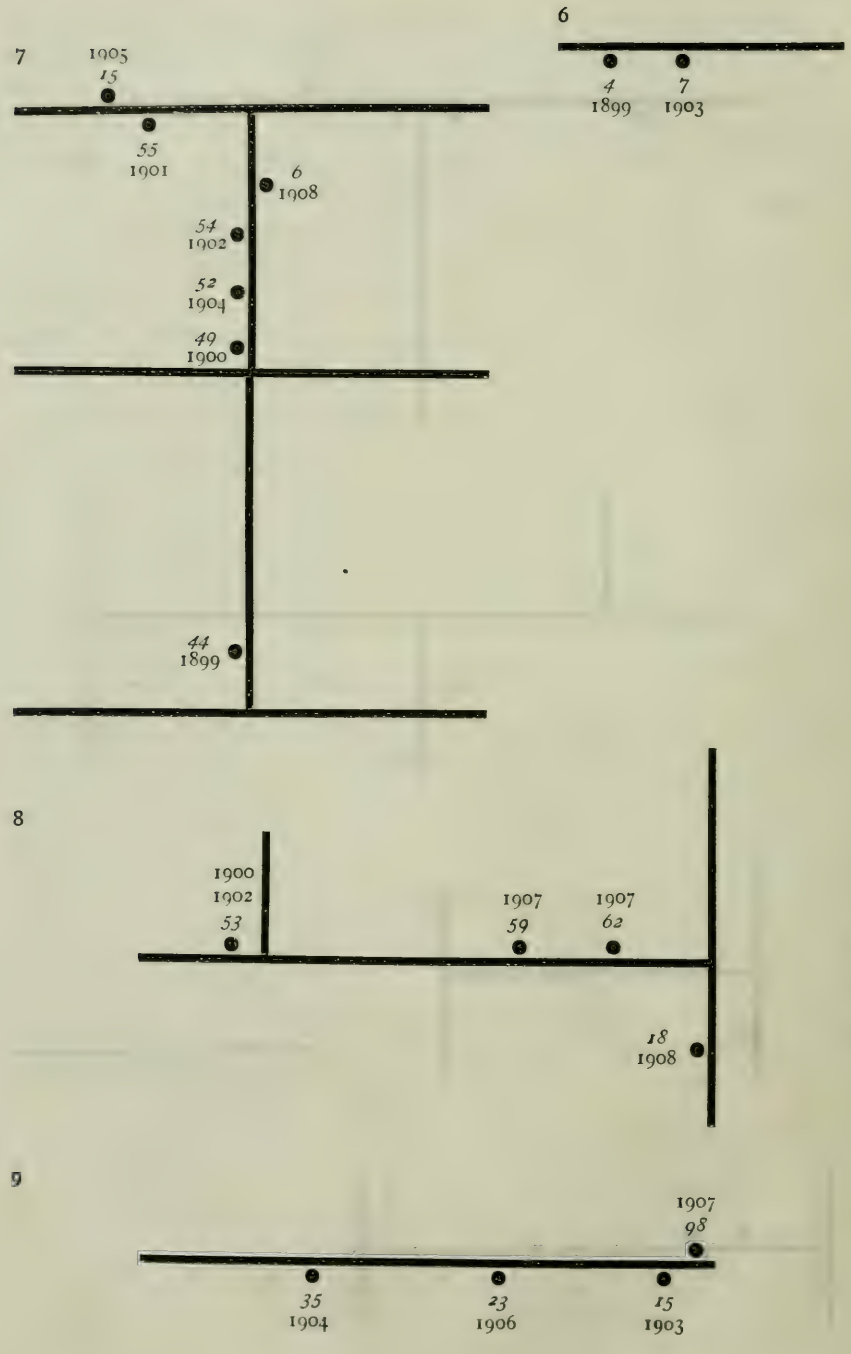
Reference to Table 1 will show that the disease prevails in Malta more than in Gozo, and that the rural population, on the whole, is more heavily affected than the urban, the east of the Island more than the west, with an intermediate zone exhibiting intermediate intensity. Endemicity is lowest in Gargur, Melleha, Floriana and Senglea; Naxaro, Valletta and Curmi come next; whilst Calcara represents the highest. Zeitun, Tarxien, Asciak, Gudia, Safi, Micabiba, Chircop, Crendi and Zurrico, a group of villages to the

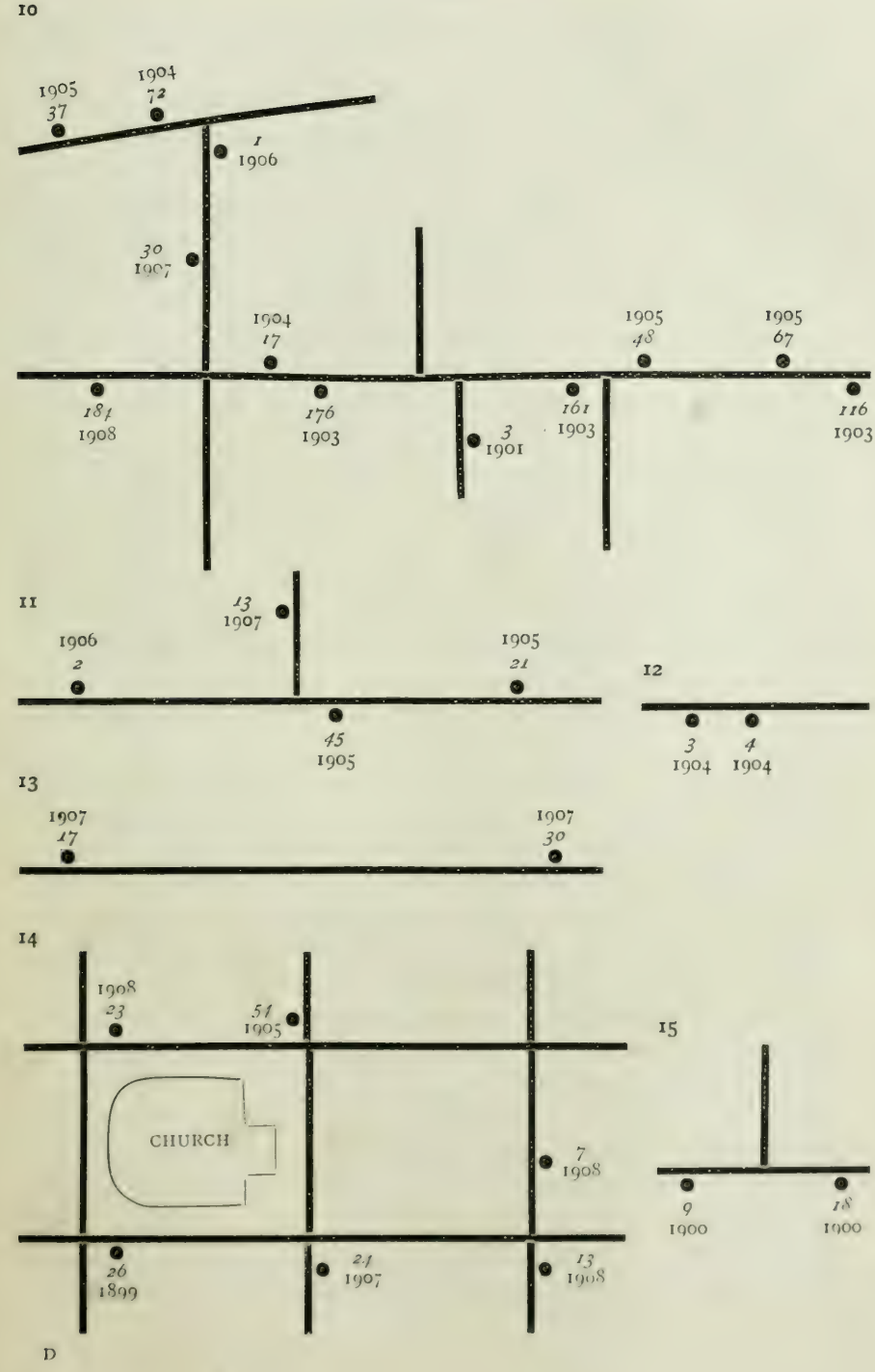
east of a line passing along the greater axis of the Island, and at no great distance one from the other, appear to suffer heavily, their indices varying from 6 to 10·9. One fact stands out: the low endemicity in the towns and the comparatively moderate endemicity in the suburban areas. To what extent this difference is attributable to certain conditions found to be more closely connected with our rural populations it would be rash to say at present. But the disease is such that less personal and domestic cleanliness, worse housing conditions, more frequent excremental pollution of soil and water, closer and more indiscriminate contact with domestic animals may very well help to spread.

Analysis of deaths, besides showing the prevalence of the disease to vary in the different populated centres, furnishes data for stating that the influence of locality is still more selective. Endemicity, in fact, is often found to be restricted to, or more intense in, some neighbourhoods or streets in preference to others. To a certain extent this may be accidental. But given the very protracted course of the disease, when the deaths do not happen to be separated by a lapse of several years, inference is justifiable that the specific virus has been conveyed from one house to the other by some common carrier. Allowing for changes of residence, which are not frequent in the villages, bringing together persons that were infected in different, and parting those that were infected in, the same streets, I believe the following graphs to be of interest. The broad lines represent roadways between blocks of buildings, the dots stand for houses from which deaths were registered, the figures in italics indicate the number of the houses, the others the year in which death occurred.

Age and sex. The total deaths under 5 for the period 1899-1908 were made up of 392 males and 352 females. Of 41 deaths at ages over 5, 16 were between 5 and 10 years of age.


1
1904
10
1902
16
44
1903
34
1901
2
26
1901
6
1908
15
1901
16
1907
3
1903
1
43
1905
4
33
1903
30
1903
5
1899
3
1899
5
30
1900
25
1899


6
4
1899
7
1903
7
1905
15
55
1901
6
1908
54
1902
52
1904
49
1900
44
1899
8
1900
1902
53
1907
59
1907
62
18
1908
9
1907
98
35
1904
23
1906
15
1903


10
1905
37
1904
72
1
1906
30
1907
1904
17
1905
48
1905
67
184
1908
176
1903
3
1901
161
1903
116
1903
11
13
1907
1906
2
1905
21
45
1905
12
3
1904
4
1904
13
1907
17
1907
30
14
1908
23
51
1905
CHURCH
7
1908
26
1899
24
1907
13
1908
15
9
1900
18
1900
D

In the succeeding table deaths have been grouped according to sex and age. (Table 2.)

Deaths under 1 appear to be equally distributed; but there is a distinct predominance of males over females at age group 1 to 2, which is responsible for nearly all the excess observed in the total males over females. Again, age-groups 1 to 2 contribute almost half the total deaths, 328, while the age-groups immediately preceding and following these account between them for 252 deaths. Owing to the protracted course of the disease, deaths recorded at ages below six months cannot be counted as caused by the disease unless it be assumed that this may prove fatal in a comparatively short time. As yet no such instances have been met with by me. Ponos, however, a disease endemic in the Greek islands of Spetsae and Hydra identical with 'marda tal biccia,' both as to its symptomatology and age of persons attacked, may prove fatal in one or two months.

Social circumstances are not specially restrictive of the disease, as cases do occur in families of well-to-do people, where the usual conditions associated with poverty are absent; its prevalence, however, appears to be more extensive among the children of the lower classes. Children born of English or Italian parents are not immune.

Recurrence of the disease in the same family and amongst relatives. Investigation has shown instances of brothers and sisters or first cousins dying of the disease to be not infrequent. Besides the cases that have come under my observation, I have been able to trace several others. These do not represent all that could be collected; but inquiry over a period reaching sometimes ten or twelve years back is for obvious reasons not easy, the more so when one has to overcome a not unnatural reticence founded on the belief that a sort of taint attaches to the disease. The view maintained by some practitioners that 'marda tal biccia' is a hereditary complaint is based on its aptitude to recur in two or more members of the same family. This standpoint is untenable both as regards direct transmission or transmission of proclivity if the following facts be duly considered, viz.: the special age incidence, the almost inexorably fatal termination of the disease, the healthiness of parents whose children are attacked, and of the

TABLE 2.—Showing for the Island of Malta the number of deaths from leucocythaemia, splenic leukaemia, splenic anaemia, pseudoleukaemia, splenitis, splenopathy under 5 and in several age groups in both sexes for the period 1899-1908, also number of deaths similarly certified during the same period for the Island of Gozo.

Locality	Sex	Age Groups									
		Under 3 months	3 months	6 months	Under 1 year	1 year	2 years	3 years	4 years	Under 5 years	Over 5 years
Malta	Males	9	12	58	80	182	76	21	9	368	—
	Females	8	11	50	70	146	68	29	5	318	—
	Total	17	23	108	150	328	144	50	14	686	41
Gozo	Males	—	—	—	—	—	—	—	—	24	?
	Females	—	—	—	—	—	—	—	—	34	?
	Total	—	—	—	—	—	—	—	—	58	?

brothers and sisters of the children attacked who very often are members of a large family.

Instances of recurrence in the same family and amongst cousins are here appended, showing sex, age at death, year and month in which they died. (Table 3.)

In Groups 1 to 12 and 18 the patient died before his or her sister or brother were born, in some cases several months intervening between the two events. The disease, therefore, cannot have been transmitted by direct contact. As it happens, belief in the communicability of 'marda tal biccia' is so rooted in the mind of the people that all articles of clothing and bedding used by the little patients are destroyed. Transmission by fomites is thus hindered to a considerable extent. Hence the existence of an intermediate parasite host becomes extremely probable. The sphere of action of an animal host would extend to members of different families in so far as their connections are more intimate and frequent. Generally speaking this is true of persons related by marriage and of their children. Instances of the disease among first cousins can thus be accounted for.

The specific cause of 'marda tal biccia' is a protozoon of the genus *Leishmania*. The parasites are morphologically identical with *Leishmania donovani*. Described first by G. Pianese, in 1905, in the splenic tissue from some cases of infantile splenic anaemia, they were observed in three cases of infantile splenomegaly in Tunis by C. Nicolle and E. Cassuto, in 1908, of which the former succeeded in cultivating the parasite and named it *Leishmania infantum*. Since then many similar observations have been made by Gabbi, Basile, Jemma, and Feletti in Southern Italy and Sicily; Sluka in Vienna; the writer in Malta*; Alvarez in Lisbon. Infantile leishmaniasis is found to have a daily widening endemicity. In common with other observers, the writer has found the Leishman bodies in the spleen, liver, kidneys and mesenteric glands, and once in films of peripheral blood. As the morphology and staining reactions of the parasite are well known, any mention here would be superfluous. Splenic puncture *intra vitam* was performed in

* Kala Azar Infantile à Malte. Note préliminaire. Archives de l'Institut Pasteur de Tunis. II. 1910.

TABLE 3—BROTHERS AND SISTERS

	Sex, and age at death	Year and month of death	Residence at time of death
1.	P.P., 2, male	Died March, 1904	Same house
	M.P., 6/12, female	,, November, 1904	
2.	R.C., 1 8/12, female	,, May, 1904	,,
	A.C., 1 1/12, male	,, July, 1905	
3.	E.P., 1 9/12, female	,, September, 1903	,,
	F.P., 10/12, female	,, January, 1907	
4.	L.M., 3, male	,, March, 1903	Different houses
	N.M., 1 4/12, male	,, March, 1906	
5.	C.C., 3, female	,, April, 1903	,,
	A.C., 2, male	,, August, 1907	
6.	C.V., 1½, female	,, August, 1901	Same house
	A.V., 1 3/12, female	,, November, 1907	
7.	C.G., 1, male	,, October, 1902	,,
	N.G., 1, female	,, February, 1905	
8.	A.P., 2½, male	,, March, 1902	,,
	C.P., 1½, male	,, April, 1904	
9.	A.X., 1 8/12, male	,, January, 1903	,,
	E.X., 1½, male	,, December, 1908	
10.	N.A., 2½, male	,, December, 1899	,,
	N.A., 2 4/12, male	,, May, 1904	
11.	M.C., 3, female	,, June, 1900	,,
	R.C., 2 1/12, female	,, November, 1902	
12.	L.D., 2, male	,, October, 1906	,,
	S.D., 1, female	,, May, 1908	
13.	G.A., 2 3/12, male	,, June, 1900	Same street, different number
	C.A., 1 2/12, male	,, July, 1901	
	R.A., 1½, female	,, October, 1903	
14.	P.C., 2 2/12, male	,, May, 1904	,,
	S.C., 4, female	,, November, 1907	
	M.C., 9/12, female	,, April, 1908	
15.	P.M., 3½, male	,, October, 1902	,,
	C.M., 2½, female	,, April, 1904	
16.	A.M., 3, male	,, July, 1907	Same house
	C.M., 1 10/12, male	,, December, 1907	
17.	M.F., 1½, female	,, October, 1901	,,
	G.F., 1, male	,, February, 1902	
18.	A.C., 1 7/12, female	,, March, 1904	,,
	R.C., 1 4/12, female	,, March, 1908	
19.	C.B., 1 8/12, female	,, September, 1899	,,
	G.B., 3, female	,, June, 1902	
20.	S.S., 3, male	,, November, 1904	,,
	E.S., 2, female	,, May, 1905	

TABLE 3—continued.—FIRST COUSINS

Sex and age at death	Year and month of death	Residence at time of death
21. S.B., 1 8/12, male	Died February, 1908	Different houses
S.B., 5, male	,, September, 1908	
22. C.M., 1½, female	,, January, 1902	,,
G.M.M., 2, male	,, July, 1907	
23. C.F., 1 10/12, female ... ... (Cousin to No. 4)	,, October, 1908	,,
24. A.C., 6½, male	,, May, 1903	Same street,
N.C., 1 7 12, male	,, February, 1903	different number
A.C., 1 4/12, male	,, December, 1903	Different street
25. A.G., 3, male ... ... (Cousin to No. 5)	,, February, 1908	Different houses
26. C.V.,10/12, female ... ... (Cousin to No. 6)	,, May, 1905	,,

five cases*: no untoward results were observed. The examination of the contents of blisters raised by vessication resorted to in two clinically typical cases of the disease proved negative. In several cases material for examination was available twenty-four hours or more after death, but the appearance of the Leishman bodies was still characteristic, only they were a little smaller than those obtained during life and their cytoplasm stained badly or not at all. The best specimens are obtained by splenic puncture *intra vitam*. The free parasites in the same film vary somewhat in size and shape, elongated and round forms are met with side by side; some have typical chromatin masses, in others the blepharoplast is punctiform, others, again, show the nucleus only. Forms are also met with containing two large chromatin masses, or nuclei, with or without a blepharoplast.

The writer found 7 out of 53 stray dogs examined post mortem in April and May, 1910, infected with *Leishmania*, sp.; 11 dogs seen in September were free. Some dogs were heavily infected, others less so, the parasites being always more numerous in the spleen than in the liver. The bone marrow was not examined. Almost all the

* In March, 1911, another case—a boy, 2 years—was diagnosed by splenic puncture.

infected dogs were small mongrels, some were mangy and extremely emaciated, one had chronic sores on the ischia and suppuration of the conjunctivae. A few ticks, of which some were gravid females off an infected dog, were dissected and examined with negative result.

Only in a few instances dogs have been found associated with human leishmaniasis, on the whole less frequently than expected. Until it be known how the virus is eliminated from the body of a naturally infected dog, and whether it may be withdrawn from its blood by blood-sucking insects, the results of my enquiries in this direction cannot minimise the importance of this animal as a probable factor in the transmission of the disease. If the excreta of an infected dog are able to carry infection or represent the means by which the parasite is dispersed about in order to undergo some as yet unknown developmental cycle, the presence of a diseased dog in a given street or neighbourhood is sufficient to explain the endemicity of 'marda tal biccia' in such street or neighbourhood.

In the light of this hypothesis the special incidence of the disease at certain ages below five, and its preference for the children of the lower classes are easy to explain.

By far the largest number of deaths occur between the 12th and 24th month of life, the next heaviest mortality is registered at ages between 24 and 36 months, the next again between the 6th and 12th months. It is not inconsistent with the variable duration of the disease for infection to occur when the child, having manifested more or less precociously a certain desire or ability to use its limbs, is put down to crawl. As long as the child is unable to do so the chances of infection appear to be very small. The families of the poorer classes, as a rule, live in the ground-floor, in the front room by preference, where they get more light and air; more often than not they have no other accommodation. The children crawl about through the doorway to the street. This ground they hold in common with the dog, that trots from door to door at its leisure. If the dog is a reservoir of the virus, its habits and the habits of children are so fitted that infection by ingestion or through skin abrasions is bound to occur.

The scope of this paper is to put on record that 'marda tal biccia' and anaemia infantum a leishmania, or infantile kala azar, are

one and the same disease, and to contribute to the study of some of the conditions associated with it.

As a specific parasitic complaint 'marda tal biccia' becomes *ipso facto* preventable.

The ease with which dogs contract experimental leishmaniosis, and the presence of infected dogs wherever infantile leishmaniosis has been shown to exist, make it extremely probable that the dog is a very important factor in the propagation and continued existence of the disease. The exact way of transmission is occupying the attention of several observers. Whether the dog be the only channel of infection, with or without the mediation of insects, or whether the disease be also contracted by one human being from another without the intervention of a lower animal, it is hoped that the epidemiology of the disease may be soon cleared up so that prophylactic measures may be applied on sound scientific lines.

I.—A RESEARCH INTO THE PRODUCTION, LIFE AND DEATH OF CRESCENTS IN MALIGNANT TERTIAN MALARIA, IN TREATED AND UNTREATED CASES, BY AN ENUMERATIVE METHOD

BY

DAVID THOMSON, M.B., CH.B. (EDIN.), D.P.H. (CAMB.)

(*Received for publication* 23 *February*, 1911)

PREFATORY NOTE.

This research has been carried on in the Tropical Ward of the Royal Southern Hospital, Liverpool, under the direction of Major Ronald Ross, C.B., F.R.S., and is a continuation of the research described in a former paper (Ross and Thomson [1910]). The funds were supplied by the Advisory Committee of the Colonial Office. The work has been facilitated by a new instrument, which enables one to estimate the number of parasites, leucocytes, etc., in a given volume of blood by a method based on Ross's 'Thick Film Process' [1903]. A following paper will describe this instrument and the method of its use.

INTRODUCTION

Knowledge regarding 'Crescents,' or the sexual forms of the malignant tertian malarial parasite (*Plasmodium falciparum*), is of considerable importance owing to the fact that mosquitos are infected by them, and thereby transmit the disease from man to man. As is well known, there are three distinct stages in the life history of the malarial parasite, namely (1) the stage of asexual parasites (fever forms); (2) the stage of sexual parasites or gametes; and (3) the stage of the parasite in mosquitos.

All these stages are essential for the spread of malaria, so that by dealing successfully with any one stage the disease can no longer be propagated, and must therefore dwindle and die.

It is, however, the second stage (sexual stage) of the parasite that I wish to consider. Less is known concerning it than of the first and second periods. No one can demonstrate how the sexual forms are produced, nor how long they live; and no effective method of killing them has been found. Research regarding this obscure stage is therefore necessary, and of great importance. When we know how to destroy the sexual malarial parasites, or how to prevent their production, we will have another powerful weapon whereby we can exterminate the disease. In this article I shall call the sexual parasites 'crescents,' as I have dealt only with cases of *P. falciparum*. The accompanying table has been compiled from the cases studied by the enumerative method used in this research. Some of the results have already been mentioned in the previous paper referred to above.

THE PRODUCTION OF CRESCENTS

From the figures given regarding the forty-two cases of *P. falciparum* studied, it is clearly noticeable that the production of crescents is extremely irregular. Certain paroxysms of fever result in a numerous brood of crescents even up to 7,000 per c.mm. of the patient's blood. Other paroxysms produce very few or none at all. Thirty-one, or 74 per cent. of the cases, showed crescents at some time during the period of examination. Eleven, or 26 per cent., showed no crescents at any time while under observation.

A. How are Crescents produced and where are they developed? All malarial experts seem agreed that the crescents are developed from the ordinary asexual spores or merozoits of the parasite.

Mannaberg [1894] stated his belief that they were produced from the conjugation of two asexual parasites within a red corpuscle. If this is so, then the more numerous these asexual forms are, the greater is the likelihood of two or more finding their way into a red cell, that is according to the theory of probability. In Case 13 where there were 300,000 asexual parasites per c.mm. of blood, many of the red cells contained more than one parasite. In Case 18 asexual parasites were few and difficult to find (1,860 per c.mm.), and no doubly infected corpuscles could be detected; yet

in the former case no crescents were produced, whereas in the latter, 286 crescents per c.mm. of blood appeared. Again, as shown in Table A, the cases with very numerous asexual parasites produced on the average fewer crescents than cases with much less numerous asexual parasites. These facts would appear to bring strong evidence against Mannaberg's hypothesis. The following quotation is from Stephens and Christophers [1908]:—'The sexual cycle, it has been thought, commences in the blood when the conditions are unfavourable for the continuance of the asexual cycle, and, in fact, has been taken as a sign that the patient has already developed immunity against the fever-producing young parasites (spores). Thus it is well known that in malignant tertian the sexual forms, gametes or crescents, first appear a week to ten days after the first febrile attack. If this view be true, then it follows that the gametes develop from forms already present in the system, viz., the asexual forms, and possibly the divergence into sexual forms takes place from the youngest form of the parasite, i.e., the spore. But it is possible that the divergence takes place at a stage previous to the youngest form of the parasite, i.e., at a stage immediately preceding the entry of the sporozoits into the blood, so that we have from the first indifferent and sexual forms present, involving indeed the existence of three kinds of sporozoits. Sexual development has been supposed to proceed mainly in the internal organs, e.g., the bone marrow, but it is being gradually recognised that young forms of gametes are also found in the circulation.'

This research would appear to support the idea that the crescents are developed from the asexual spores when a certain amount of immunity has developed, but it seems to me that they do not come from special asexual spores, but that they arise merely owing to a transformation of an ordinary asexual spore into a sexual parasite. I will give evidence to show that the development of immunity is necessary for their production, and I fail to see why, if they do develop from special spores they cannot be produced at any time independently of immunity. There seems to be little doubt also that they develop chiefly in the internal organs, and when completely developed they appear suddenly in the peripheral blood; for, although small undersized crescents are sometimes seen, yet their occurrence in the peripheral blood is rare. I have never seen

in the peripheral blood anything which could be considered as an intermediate stage between an asexual spore and a true crescent.

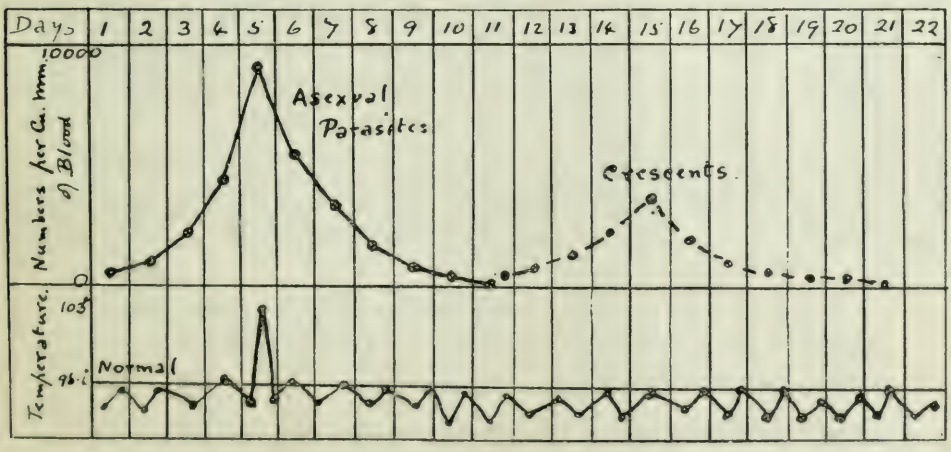
B. When are Crescents produced? The accumulated evidence of the crescent cases studied seems to show that a period of ten days elapses between the appearance in the peripheral blood of the asexual spores, and the crescents which are produced from these spores. As a general rule the crescents appear in the peripheral blood on the fifth day after the attack of fever, and increase in number for four or five more days, so that they are most numerous on about the tenth day after the height of the fever. Those crescents appearing on the fifth day after the paroxysm correspond to asexual parasites existing in the blood five days previous to this paroxysm. Asexual parasites can exist in the blood in numbers as great as 2,000 and rarely 10,000 per c.mm. without producing any temperature reaction. They gradually increase in number by sporulation till they are numerous enough to cause a paroxysm of fever. The numbers may then fall spontaneously or by quinine treatment so that only one single paroxysm results. It is by the study of such single paroxysms that the time required for the appearance in the peripheral blood of the corresponding crescents can be best determined. In such cases the graphs representing the numbers of asexual parasites and crescents show a striking similarity, the points on the crescent graph occurring on the tenth day after the corresponding points on the graph of the asexual parasites. It is very difficult to demonstrate this with mathematical accuracy, and very frequent examinations of the blood require to be made.

The chart of Case 20 shows the correspondence fairly well. A careful study of all the charts leads one to the conclusion that the corresponding points on the crescent curve occur about ten days after those of the asexual curve. This conclusion is strengthened by the chart of Case 38. In this case the numbers of leucocytes, crescents and asexual parasites were estimated several times daily for twenty-three days.

This case is extremely interesting, as the patient had nine successive daily paroxysms of fever. Four paroxysms on the four days before admission, and five paroxysms on the next five days. Corresponding to these nine fever paroxysms or sporulations we

have nine outbursts of crescents into the peripheral circulation, each occurring on the tenth day after the corresponding fever paroxysm. The asexual parasites were rapidly destroyed by quinine after the ninth paroxysm of fever, and a corresponding diminution in the production of crescents is evident ten days later. It is stated in the above quotation from Stephens and Christophers that many have observed the appearance of crescents on the eighth to the tenth day after the first paroxysm of fever, but this delay is attributed to the development of immunity, whereas it is due to the fact that crescents take that time to develop from the asexual spores before they appear in the peripheral circulation. The following diagrammatic charts A and B represent the correspondence between asexual parasites and the crescents developed therefrom, where only one blood count is made per day.

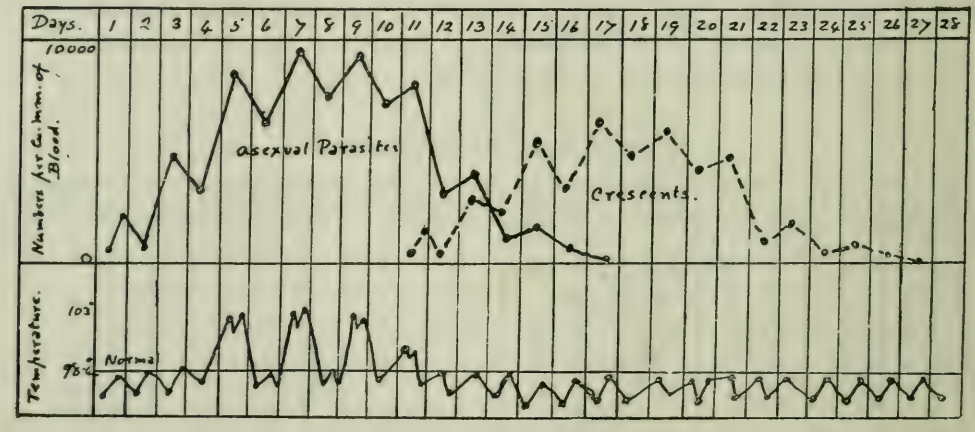
DIAGRAM A. (Single fever paroxysm.)



C. WHY ARE CRESCENTS PRODUCED IN SOME CASES AND NOT IN OTHERS? Crescents would seem to be developed from the asexual spores, due to a development of immunity towards the latter. When the asexual spores find that their environment is becoming unsuitable, they undergo a transformation into a sexual generation and thereby save themselves from destruction. In this new state they remain passive, waiting for their transference into a more suitable host. Schaudinn and other observers have stated that

they have seen sexual gametes undergoing a change back into asexual spores by parthenogenesis. Although this has never been observed by workers in the Liverpool School of Tropical Medicine, yet in the light of the above theory it would seem quite possible that such a retransformation might take place, especially in cases where the acquired immunity had become less or disappeared. This phenomenon must, however, be very rare, as it would otherwise have been noticed more often.

DIAGRAM B. (Showing tertian paroxysms with corresponding outbursts of crescents ten days later.)



The following evidence would seem to supply seven points in support of the statement that crescents are formed after the development of partial immunity.

(1) *The relationship between the number of asexual parasites and the number of crescents produced.* Taking very acute cases, I find that out of eight paroxysms of fever caused by numbers of asexual parasites over 50,000 per c.mm. of blood, only three, or 37·5 per cent. resulted in crescent production. A total of 724,000 asexual parasites per c.mm. produced a total of 1,354 crescents per c.mm., giving a ratio of 535 asexual parasites to one crescent.

Eleven subacute paroxysms had asexual parasites varying from 20,000 to 50,000 per c.mm. of blood; of these, seven, or 63·6 per cent., resulted in crescent formation. A total of 318,700 asexual

parasites per c.mm. produced a total of 3,952 crescents per c.mm. of blood, giving a ratio of 81 asexual parasites to one crescent.

Twenty-six mild chronic cases had asexual parasites, varying from 1,100 to 20,000 per c.mm. of blood. Eighteen of these, or 69·25 per cent., resulted in crescent production. A total of 172,360 asexual parasites per c.mm. produced a total of 3,343 crescents per c.mm. of blood, giving a ratio of 52 asexual parasites to one crescent.

The mild, chronic, and probably partially immune cases had therefore a crescent-producing power fully ten times greater than the very acute cases.

(2) *The duration of the disease in relation to the production of crescents.* Sixteen paroxysms occurred during the first thirty days of the disease. Of these 43·7 per cent. produced crescents. The total average number of crescents was 161 per c.mm. of blood.

Twenty paroxysms occurred between the thirtieth and the sixtieth day of the disease. Of these 65 per cent. produced crescents, the total average number of crescents being 551 per c.mm.

Sixteen paroxysms occurred after the sixtieth day of the disease. Twelve, or 75 per cent. of these produced crescents, and the total average number of crescents was 650 per c.mm. of blood.

Thus it would appear that crescents are more likely to be produced in cases of long standing, where a certain amount of immunity has had time to develop.

(3) *Crescent production in cases which have had previous attacks of fever one or more years previously.* Sixteen cases had had previous attacks of fever. Of these, 87 per cent. developed crescents during the period of observation. There were twenty-six cases which had no history of previous attacks; of these, only 46 per cent. produced crescents. It is reasonable to suppose that these cases, which had a history of previous attacks, were more immune than the primary cases.

(4) *The relationship between crescent production and the age of the patient.* From the table it can be seen that 50 per cent. of the cases up to twenty years of age (average eighteen years) produced crescents, the average number being 130 per c.mm. of blood.

Cases between twenty and thirty years of age (average twenty-six years) gave an average of 526 crescents per c.mm., 74 per cent. of the paroxysms resulting in crescents.

Cases between thirty and sixty-eight years of age (average forty-five years) gave an average of 1,018 crescents, 71 per cent. of the paroxysms producing crescents.

There is apparently an increase in crescent-producing power in older patients. This might be attributed to a greater power in adults of developing immunity, as compared with the young growing patients. Many of the older patients, however, had been in the tropics for a long time, and had had previous attacks of fever. The young patients had not been long in malarial districts, and in most cases it was their first attack of malaria. The greater crescent-producing power in the older patients may therefore have been due to immunity developed from previous attacks.

(5) *The relationship between crescent production and the percentage of the patient's haemoglobin.* Nineteen paroxysms of fever, occurring chiefly in different cases where the haemoglobin during the next ten days was 75 per cent. and under, produced only an average of twenty-two crescents per c.mm. of blood. Of these paroxysms, 52 per cent. produced crescents.

Twenty-seven paroxysms, where the haemoglobin during the next ten days was over 75 per cent., produced an average of 428 crescents per c.mm., and nineteen, or 70 per cent., of these paroxysms produced crescents.

It would seem therefore that a low percentage of haemoglobin is not so favourable for crescent production as a fairly high percentage. This again might be explained by the supposition that immunity to the asexual parasites is more likely to be successfully developed in cases where the blood standard is fairly healthy.

(6) *Crescent production in relation to the size of the spleen.* Twenty-six cases had palpable spleens. Of these, fifteen, or 75 per cent., produced crescents. In twenty-three cases the spleen could not be palpated. Twelve, or 52 per cent. of these produced crescents.

According to Ross [1910] the number of asexual parasites tends to vary inversely as the degree of splenomegaly, that is, the parasites tend to die out in persons with very large spleens. Again, N. F. Surveyor [1910] states that malignant malaria is more fatal in cases where the spleen is not enlarged, and less fatal in those with splenomegaly. These statements would seem to indicate that immunity to the disease increases *pari passu* with the size of the spleen; hence the increased crescent production where the spleen is enlarged.

(7) *Crescent production in relation to the number of leucocytes.* The average number of leucocytes in the ten-day periods following paroxysms which produced no crescents was 7,284 per c.mm. of blood (56 per cent. mononuclears). After paroxysms producing up to 100 crescents per c.mm., the average number of leucocytes during the same period was 7,411 per c.mm. (52 per cent. mononuclears). While after paroxysms producing over 100 crescents per c.mm., the average number of leucocytes was 8,924 per c.mm. (53 per cent. total mononuclears).

Again in thirteen cases, which produced no crescents at any time, the average number of leucocytes throughout was 8,646 per c.mm. (total mononuclears 55·9 per cent.), as compared with an average of 10,970 per c.mm. (total mononuclears 48·9 per cent.), in sixteen cases with numerous crescents throughout the period of examination.

It would appear therefore, that greater numbers of crescents are produced in cases where the leucocytes are numerous.

The leucocytes in malaria increase markedly in number, simultaneously with the quiescence of the disease. About one week after the last paroxysm of fever, the leucopenia (characteristic as a rule of the febrile period in malaria) disappears, and a leucocytosis takes its place, provided the fever does not return. Thus a high leucocyte count is characteristic of quiescent malaria and in post-malarial conditions, and would appear to be coincident with periods of immunity. This increase in the number of peripheral blood leucocytes occurs after the fever abates, whether quinine has been given or not. Where quinine is given, it occurs earlier and remains permanent, because during the treatment no true relapse

can occur. The leucocytes decrease in number previous to the onset of a relapse. The chart of Case 20 shows the fall in the number of leucocytes with the onset of a relapse, and later an increase in crescent production when the leucocyte count again becomes high. These facts would tend to show that a high leucocyte count and immunity are co-existent, the latter explaining the increase of crescents.

(8) *Crescent production in relation to the month of infection.* We have not sufficient cases on record to make any reliable deductions. We can only state, that from West Africa, cases infected in all months of the year except June produced crescents, and again cases infected in every month, except May and July, showed no production of crescents. From this evidence there seems no reason to suspect that crescent production depends upon the month of infection.

D. The Effect of Quinine on Crescent Production*. It would appear that quinine in doses of ten grains three times daily, given just before and during the paroxysm of fever, diminishes the subsequent formation of crescents. The cases showing the greatest numbers of crescents had little or no quinine for several days previous to the producing paroxysm, and little or no quinine during that paroxysm. Case 23 seems to show the good effects of quinine in this respect. In this case the first paroxysm of fever produced 852 crescents per c.mm. of blood, resulting from 50,000 asexual spores per c.mm. Twenty grains of quinine were given during this paroxysm, but none was given for several days before or after it. The next relapse where no quinine was given till the day after the paroxysm produced 468 crescents per c.mm., while the next paroxysm of this relapse produced 344 crescents per c.mm. from 54,000 fever forms per c.mm. During this last paroxysm and afterwards thirty grains of quinine was given daily. A subsequent relapse treated similarly with quinine gave a production of only four crescents per c.mm. from fever forms amounting to 16,000 per c.mm. of blood. This case and others would seem to indicate that if quinine is given in doses of ten grains three times daily during

* The various salts of quinine used were kindly supplied by Messrs. Burroughs, Wellcome & Co.

the fever paroxysm and afterwards, it helps much to prevent the formation of crescents. The destructive action of quinine in these doses on the asexual spores is so powerful and rapid that one is surprised at the subsequent appearance of even a few crescents. If quinine is withheld till one or two days after the fever paroxysm and then given in the above daily doses, the crescents may still appear in large numbers—*vide* Cases 20, 22, etc. This shows that when once the crescents have commenced to develop, the quinine does not then prevent them from reaching maturity and appearing in the peripheral blood on the tenth day or thereabout. I would therefore conclude that quinine in large daily doses, given during and after the fever paroxysm, diminishes the crescent-producing power of that paroxysm, not by acting on the crescents themselves, but indirectly by destroying the asexual spores from which the crescents are produced. However, if quinine be given in smaller doses, say five grains daily, or ten to twenty grains irregularly, then instead of the crescent production becoming less, there is evidence to show that it may become even more prolific. Thus in Case 18, crescents became much more numerous after quinine was administered in daily doses of five grains. This case with such treatment showed a very high crescent-producing power, the ratio of asexual spores to crescents being as eight is to one approximately. It is quite reasonable to suppose that in such cases, quinine given in small doses destroys only some of the asexual spores, but enables the host to keep the disease under control, and to develop some resistance or immunity. In the consequence more crescents develop from the remaining parasites. Case 18 and others showed the presence of asexual parasites for many days during the administration of quinine in doses of five grains daily.

Quinine given continuously in daily doses of twenty to thirty grains, has never failed in our cases to reduce the crescents to numbers less than one per c.mm. of blood in a period not exceeding three weeks, vide chart of Case 49. This reduction of crescents by quinine has also been noted by Darling [1910].

E. The effect of Methylene Blue on Crescents. It would appear from the careful investigation of six cases treated alone with methylene blue, that this drug in doses of twelve grains daily, given by mouth (pill form), though not so potent in destroying

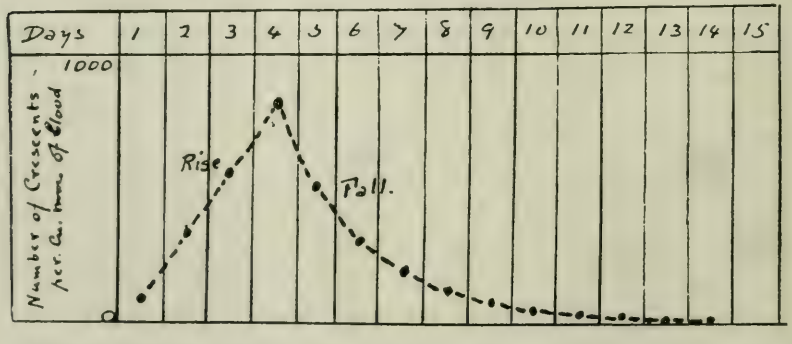
the asexual parasites, is yet more potent than quinine in preventing crescent formation. It would seem also to have some direct destructive effect on the crescents. It is good treatment, therefore, to give methylene blue along with quinine, especially where one cannot give large doses of the latter due to the idiosyncrasy of the patient.

THE DURATION OF LIFE, AND THE DEATH OF CRESCENTS

(*a*) *There is evidence to show that the duration of life of a crescent cannot be more than twenty days.* During the first ten days of this period they are developing somewhere, but not in the peripheral blood. They then appear in the peripheral blood, sometimes small in size. The majority of them must perish in the peripheral blood in a very few days. This must be so, for if they lived for say four days or longer, then the summit of the crescent curve would not be a sharp point as it always is, especially when the numbers are great.

(*b*) *The appearance of the graph, representing the life and death of crescents in the peripheral blood.* The crescent curve has a definite formation. Where the number of crescents is estimated only once daily, it assumes more or less the appearance shown in the diagram C.

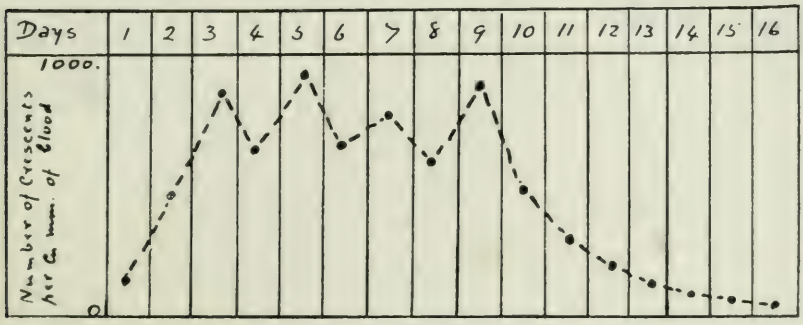
DIAGRAM C. (Crescent curve, numbers estimated once daily.)



This is the usual type of crescent curve obtained after a single isolated paroxysm of fever, where the number of crescents is estimated once daily. When no quinine is given, a single isolated paroxysm is rare, and the crescent curve will as a rule be quite

different, as in Cases 1, 14, 16, 18, 23, 24, etc. In these cases the number of crescents remains high for some days, the graph resembling a kind of plateau containing several sharp peaks, as in diagram D.

DIAGRAM D. (Crescent curve with plateau formation.)



The explanation of this plateau is quite simple, for if no quinine is given the asexual parasites remain alive, even though there is no fever to indicate their presence, and keep on producing new crescents. The source of crescents is not cut off, so that the supply is replenished by new broods of crescents, appearing every day, or on alternate days, or irregularly, according as the fever or asexual sporulation occurs every day, on alternate days, or irregularly. The sharp peaks on the plateau of the curve show that although the crescents are dying rapidly, yet their numbers are replenished by fresh broods coming into the circulation. As pointed out by Ross and Thomson [1910], these peaks on the crescent curve often show a tertian tendency.

In no case is there a plateau formed when quinine has been given in doses of thirty grains daily ten days previous to the height of the crescent curve. If a plateau has formed and quinine is then administered in large doses, its effect will not be manifested for about ten days, because although it very quickly reduces the source of supply, yet those crescents which commenced to develop during the previous ten days are not affected, but continue to appear in the peripheral blood replenishing the loss. Thus quinine, as is clearly seen in Case 23, takes about ten days to destroy the plateau formation. Hence, though quinine makes the crescents disappear from the peripheral blood more quickly than they would otherwise

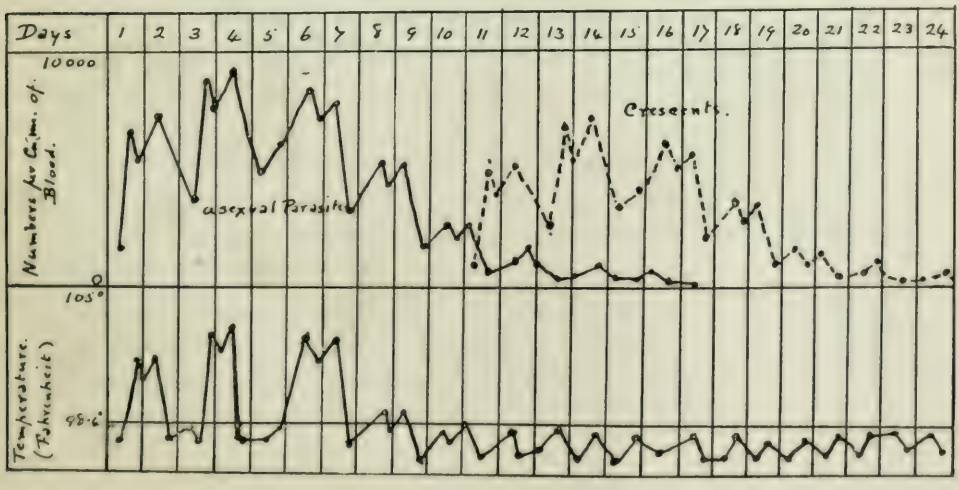
do without its administration, yet this effect is not due to any direct destructive action on the crescents themselves. It is due indirectly to the destruction of the asexual spores from which the crescents are developed. The length of time that crescents will remain in the peripheral blood therefore depends upon the persistence of the asexual parasites. If immunity develops so strongly that the asexual parasites almost disappear, or if they are destroyed by quinine, then the crescents also will disappear in due course.

In cases where immunity remains, but is only sufficient to keep the number of asexual parasites in check, crescents may continue almost indefinitely. Crescents have been observed to continue in the peripheral blood for eight weeks, Surveyor [1910]. Case 18, where the asexual source of supply was not destroyed by quinine till late, had crescents for forty-four days, and probably longer than this, as they were present when the case first came under our observation. Sufficient has been said to point out the fallacies regarding the duration of life of crescents. I must, however, once more refer to the chart of Case 38. Here the number of crescents per c.mm. of blood was estimated several times daily. The crescent graph obtained shows the great importance of making numerous observations, for had the numbers been estimated only once a day, the daily variation in the number of crescents would not have been noticed. Here we have a pure quotidian case of fever, resulting in quotidian outbursts of crescents into the peripheral circulation, each crescent outburst corresponding to a sporulation of the asexual parasites occurring ten days before. It is noticeable that the quinine in doses of thirty grains daily did not appreciably diminish the numbers of crescents till the tenth day after its administration. The number of crescents then diminished, rapidly at first and afterwards more slowly, for nine more days. This would seem to indicate that the quinine quickly destroyed the majority of the parasites of the asexual source, the remainder dying more slowly. The curve also clearly shows that the crescents die very quickly in the peripheral blood stream; a very marked fall occurs each day, but this fall is compensated for by a fresh brood each day. It is clear that but for this compensation the crescents would only remain in the peripheral blood for a very few days. Again it will be observed that although asexual parasites could no

longer be detected in the blood after the third day of quinine, yet crescents continued to be present till the eighteenth day of quinine treatment. Thus those crescents found after thirteen days of quinine administration had either a life of five days in the peripheral blood, or else they were new crescents produced from surviving asexual spores, so few in number that they could not be detected. When quinine has been given in the above doses for a few days asexual parasites can no longer be found, but the fact that relapses occur (even when there are no crescents), shows that they were still present. They may exist in numbers below the detectable limit, or (?) as resisting forms in the internal organs, and it is possible the crescents found more than thirteen days after the administration of quinine come from these.

In other cases, where the number of crescents was estimated several times daily, the graph obtained was much more irregular than in Case 38. In these, daily irregular variations took place, vide chart of case 49. This is easily explained, for in many cases of malignant tertian, sporulation is extremely irregular. It is very seldom that one gets so well a defined quotidian sporulation as in Case 38. In cases of malignant tertian therefore with irregular temperature indicating irregular sporulation, one would expect the crescent graph, as estimated from several counts daily, to be irregular also. The majority of our cases have shown this irregularity. The following diagrammatic chart will indicate the idea more clearly.

DIAGRAM E. (Represents a pure tertian fever or sporulation.)



This chart represents the true relationship between crescents and the asexual spores in a pure case of malignant tertian fever. A case with an irregular fever, indicating irregular sporulations, would give an irregular crescent graph. These points, however, still require to be worked out more thoroughly.

There is, however, one very constant law regarding the crescent graph, viz., that the curve representing the diminution or death rate of the crescents is always a parabolic line (vide curve shown in Diagram C). This shape of curve arises from the fact that they die off by a constant fraction, say one-half to one-fifth of their daily number. One might give two explanations of this law regarding their death rate.

(1) That it is a case of the survival of the fit, a certain proportion dying day by day according to their varying powers of resistance.

(2) That it depends upon the law of probability regarding their contact with leucocytes, by which they are ingested. The mononuclear leucocytes, especially the large forms, undoubtedly ingest crescents, either when alive or after their death, for in cases where only numerous crescents are present in the blood, many pigmented mononuclear leucocytes are to be found. It is quite reasonable to suppose that there may be some truth in these two hypotheses, but I think the true explanation is to be found by studying the curve of the asexual forms from which the cresents arise. It is clearly seen from the charts of Cases 17, 38, and others, that the curve representing the death rate of the asexual parasites is also a parabolic line, as shown diagrammatically in Chart A. The curve is the same, whether they die off spontaneously or under the influence of quinine. Now, when the producers of crescents show this death rate curve, and if the crescents produced have approximately all the same duration of life, then necessarily the curve of their death rate which will occur ten days later will assume the same form. Hence the peculiar form of the crescent curve depends probably in every respect upon the form of the curve of the asexual parasites. The form of the asexual parasite curve most probably depends in its turn upon (1) The law of the survival of the fit; (2) The law of probability of leucocyte contact and ingestion. The large mononuclears undoubtedly ingest the asexual parasites, especially the spores. When the spores are

numerous many will be ingested, but as they become fewer the chance of contact and ingestion by these leucocytes will become less.

CONCLUSIONS REGARDING PROPHYLAXIS

From the above research one might give the following deductions regarding the prevention of malaria:—

(*a*) It is a bad practice to give quinine in small doses of five grains daily, or irregularly, even though the doses be larger, for such treatment tends to increase the power of crescent formation.

(*b*) All cases of malaria should be treated early and continuously with doses of quinine of about twenty to thirty grains daily, as such treatment during and after the fever diminishes the subsequent formation of crescents. Continuous treatment with the above doses has never failed, as stated above, to reduce the number of crescents to less than one per c.mm. of blood in a period not exceeding three weeks. That is to say, it renders infective cases of malaria non-infective to mosquitos in a period not exceeding three weeks.

SUMMARY

1. Crescents are produced from the ordinary asexual spores of *P. falciparum*, due to a development of immunity towards the latter.

2. They develop somewhere in the internal organs and then appear suddenly in the peripheral blood.

3. The period required for their development is about ten days.

4. Crescents do not generally live more than a few days in the peripheral blood.

5. Crescents may be present in the peripheral blood during periods as long as eight weeks, not because the individual crescents survive for that time, but because their numbers are constantly replenished from surviving asexual forms.

6. Fresh broods of crescents come into the circulating blood daily, or every other day, or irregularly, according as the asexual sporulations occurring ten days before were quotidian, tertian, or irregular.

7. Quinine has no direct destructive action on crescents, either during their development or afterwards, but it destroys the asexual source of supply.

8. Quinine reduces the crescents to numbers less than one per c.mm. of blood within three weeks, provided it be given in daily doses of twenty to thirty grains.

9. Quinine in small doses tends to increase crescent production (?) by favouring the development of immunity to the asexual parasites.

10. Methylene blue in doses of twelve grains daily reduces the number of crescents, and would seem to have some direct destructive action upon them.

LITERATURE

DARLING (1910), *Ibid.* Vol. IV, No. 2.

MANNABERG (1894), 'The Parasites of Malaria Fever,' Marchiafava and Bignami, and Mannaberg, New Sydenham Society, 1894, p. 289.

ROSS (1903), 'The Thick-Film Process for the Detection of Organisms in the Blood,' Thompson Yates and Johnston Laboratories' Reports, Vol. V, part I, 1903.

ROSS (1910) 'Prevention of Malaria,' p. 129. A. Celli, p. 406.

ROSS and THOMSON (1910), 'Some Enumerative Studies on Malarial Fever,' Annals Trop. Med. and Parasit., Vol. IV, p. 267.

STEPHENS and CHRISTOPHERS (1908), 'The Practical Study of Malaria and other Blood Parasites,' p. 52.

SURVEYOR (1910), 'Some Observations on Malaria in relation to Splenic Enlargement and the Treatment of the Crescentic Stage,' *Ibid.*, Vol. IV, No. 3.

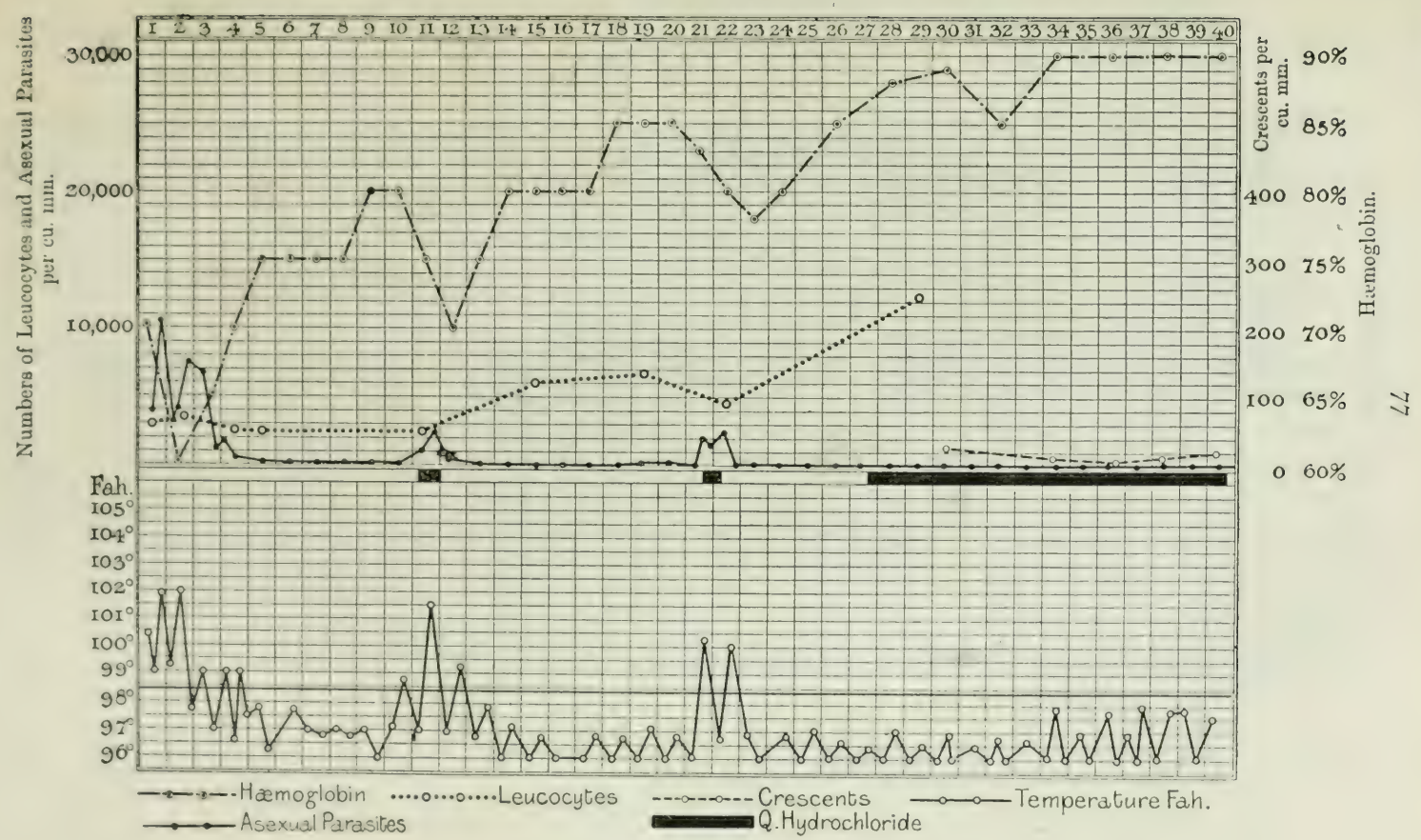
TABLE A

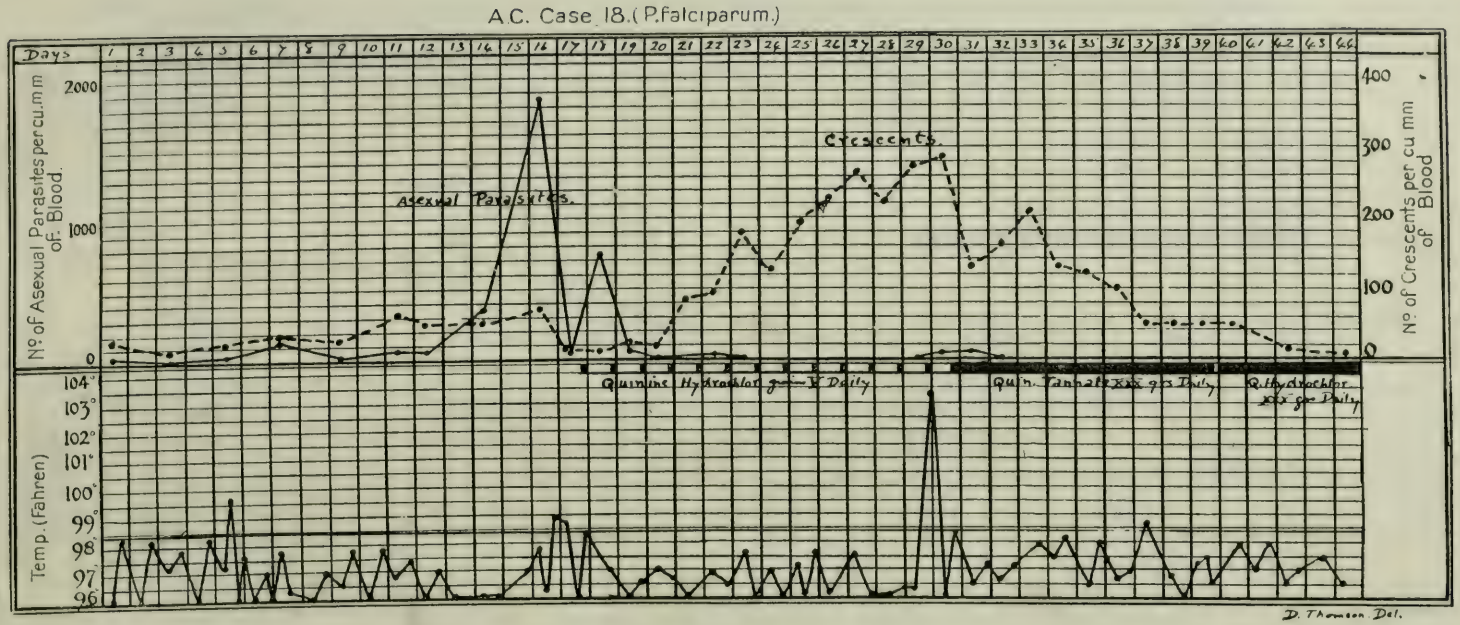
Case	Age	Where infected	Month of infection	Duration of disease up to fever paroxysm	Quinine previous to fever paroxysm	Maximum asexual parasites per c.mm. during fever paroxysm	Maximum Cresents per c.mm. produced	Day of maximum Cresent production	Average haemoglobin % during the 10-day period	Average leucocytes per c.mm. during the 10-day period	Average total mononuclear percentage during the 10-day period	Average daily quinine for 10-day period	Average daily methylene blue for 10-day period	Total duration of Cresents in days
				days								grains	grains	
10	37	Kamerun, W. A.	September	140	Nil for 3 days	15,000 ?	10	—	—	5,500	—	4	Nil	?
1	42	Niger, W.A.	November	70	Little ?	??	220	—	—	2,900	50·5	Nil	7	over 22
11	17	Niger, W.A.	January	190	Nil for 14 days	64,000 ?	0	—	—	5,600	51	30	Nil	?
12	24	Niger, W.A.	December	30	Nil for 3 days	50,000	34	10th	—	6,910	51	13	Nil	over 13
13	22	Congo	December	30	70 grains in 30 days	300,000	0	—	—	11,400	55	27	0	—
14	31	Gold Coast	November	63	Nil for several weeks	37,000 ?	1,500	—	—	7,070	43	3	0·9	Over 18
15	27	Vera Cruz	November	49	Quinine irregularly	?	7,000	—	—	12,000	54	?	0	Over 12
16	17	Niger, W.A.	September	70	Little	?	244	—	—	10,310	69	0	x c.c. polychrome blue	Over 28
17	19	Congo	January	49	Nil for 14 days	10,000	0	—	72	2,375	69	0	0	—
17 Relapse	19	Congo	January	59	Nil for 25 days	3,000	0	—	80	5,620	51	2	0	—
17 Relapse	19	Congo	January	69	Nil for 10 days	2,500	25	10th	83	8,280	44·7	15	0	—
18	29	Congo	January	77	Nil for 50 days	1,860	286	8–12th	84	9,830	54·5	4·5	0	Over 43
19	20	Niger, W.A.	February	40	Very little	5,240	0	—	72	4,300	46·6	2	0	—
20	24	Niger, W.A.	February	40	Very little	7,400	16	9th	72	5,890	39·6	3	0	33
20 Relapse	24	Niger, W.A.	February	60	Nil for 16 days	4,000	88	10th	68	4,140	—	28	0	33
21	50	Niger, W.A.	March	28	250 grains	36,000	4	—	66	4,400	—	11	0	17
22	30	Niger, W.A.	March	40	Nil for 4 days	7,920 ?	1,388	10th	80	11,550	—	22	0	31
22—2nd Parox.	30	Niger, W.A.	March	42	Nil for 6 days	45,000 ?	1,240	9–10th	78	8,850	—	30	0	31
23	17	Niger, W.A.	March	21	Nil for 14 days	50,000	852	10th	80	4,360	56	3	0	32
23 Relapse	17	Niger, W.A.	March	30	Nil for 8 days	34,000	468	9–10th	77	6,440	59	23	0	32
23 Relapse	17	Niger, W.A.	March	32	Nil for 10 days	54,000	344	10th	79	5,950	59	23	0	32
23 Relapse	17	Niger, W.A.	March	46	Nil for 7 days	16,700	4	—	82	7,780	—	30	0	32
24	29	Niger, W.A.	March	28	Nil for 9 days	25,000	572	10th	76	8,420	—	2	0	35
24 Relapse	29	Niger, W.A.	March	34	Nil for 5 days	9,500	630	10th	78	8,570	—	1	0	35
24 Relapse	29	Niger, W.A.	March	46	Nil for 9 days	4,000	68	—	79	5,500	—	22	0	35

TABLE A—continued.

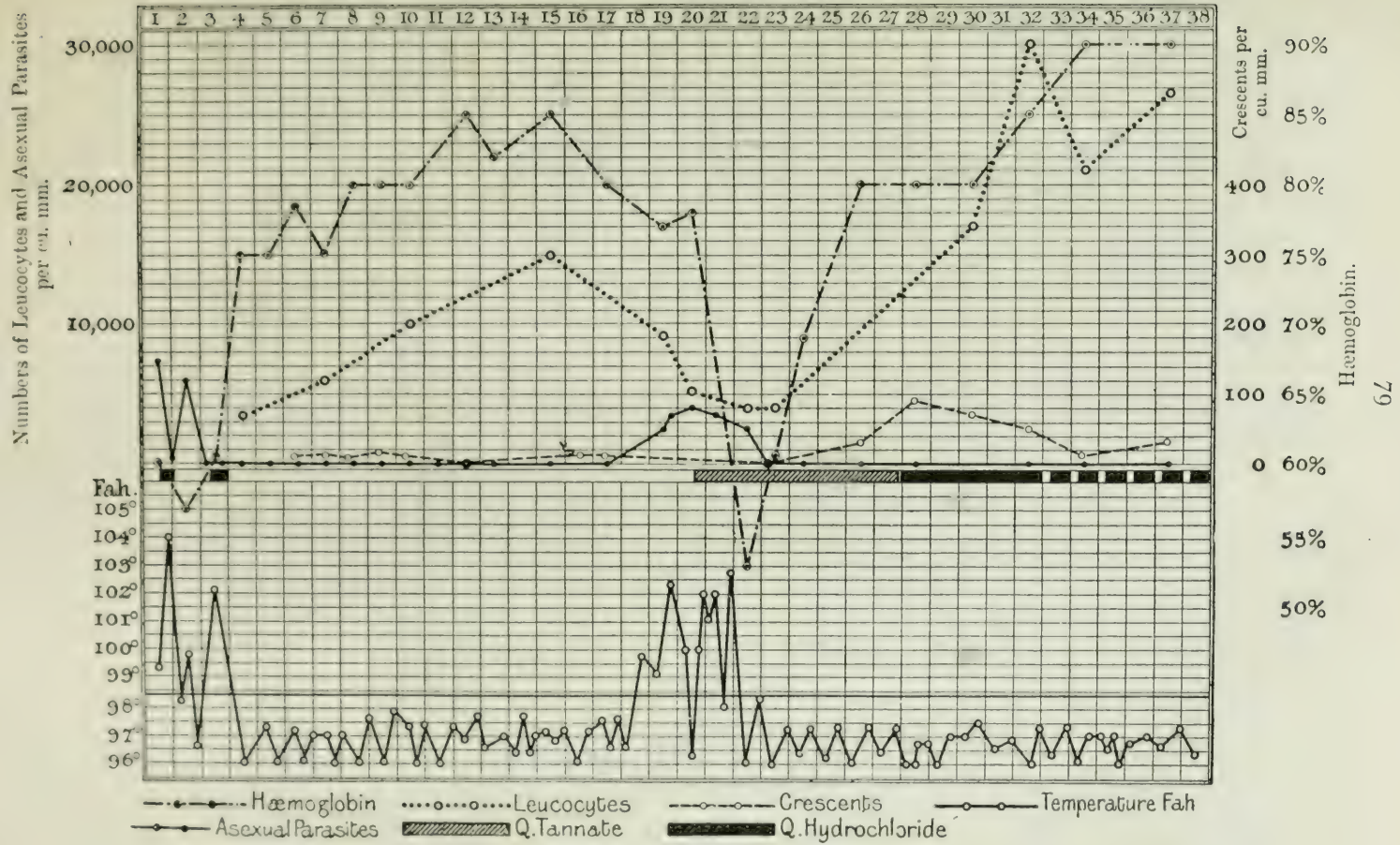
Case	Age	Where infected	Month of infection	Duration of disease up to fever paroxysm	Quinine previous to fever paroxysm	Maximum asexual parasites per c.mm. during fever paroxysm	Maximum Cresents per c.mm. produced	Day of maximum Cresent production	Average haemoglobin % during the 10-day period	Average leucocytes per c.mm. during the 10-day period	Average total mononuclear percentage during the 10-day period	Average daily quinine for 10-day period	Average daily methylene blue for 10-day period	Total duration of Cresents in days
				days								grains	grains	
24	29	Niger, W.A.	March	49	Nil for 10 days	15,000	28	—	82	6,580	—	30	0	3
Relapse														
25	20	Gambia	March	23	Nil for 6 days	22,000	0	—	77	8,200	—	27	0	—
26	44	Niger, W.A.	April	20	Little	9,700	0	—	85	10,540	—	10	0	—
26	44	Niger, W.A.	April	27	Nil for 7 days	2,000	0	—	88	15,650	—	30	0	—
Relapse														
27	30	W.A.	May ?	?	Nil for weeks	1,104	8	—	74	12,320	—	0	0	Over 4
28	25	Niger, W.A.	April	42	Nil for 7 days	3,040	0	—	87	8,190	58	9	0	—
29	38	Niger, W.A.	April	70	Nil for 2 days	6,500	4	—	63	7,940	61	25	0	—
30	53	Niger, W.A.	May	15	A little	4,000	152	9th	90	8,940	57	1	0	Over 12
31	20	Niger, W.A.	March	90	10 grains daily for 10 days	28,000	0	—	75	5,170	59·3	0	10	—
32	20	W.A.	February ?	150	Nil for long time	few	244	—	76	11,710	60	0	0	Over 7
33	18	W.A.	June	10	?	6,700	0	—	99	7,410	57·5	14	0	—
34	23	W.A.	August	63	Nil for 3 weeks	84,400	0	—	69	6,159	50	27	0	—
35	16	W.A.	October	14	Nil for 7 days	70,000	0	—	34	8,540	52	20	0	—
36	38	W.A.	October	9	Little	50,000	0	—	80	3,200	62	25 5	0	—
37	26	W.A.	September	21	Nil for 5 days	29,000	0	—	78	10,040	77·3	19·5	0	—
38	25	W.A.	July	91	Very Little	26,900	144	10th	80	11,350	38·5	16·5	0	22
39	40	W.A.	September	70	Nil for 6 days	22,000	24	10th	49	5,830	57	26	0	Over 11
40	19	W.A.	September	24	Nil	21,800	0	—	62	9,240	80, only one count	0	12	—
41	24	Southern U.S.A.	October	49	Nil for 2 days	20,000	0	—	45	5,450	45·2	10·5	11	Over 5
41	24	Southern U.S.A.	October	54	Nil for 6 days	12,000	0	—	50	6,050	43	17	12	Over 5
Relapse														
42	28	U.S.A.	October	49	Nil for 21 days	7,000	48	9th	58	10,820	45	0	0	Over 20
43	22	W.A.	August	21	Nil for 3 weeks	6,000	500	—	93	10,600	49·5	22	0	Over 16
44	26	W.A.	September	42	Nil for 5 days	5,400	32	9th	79	7,800	—	30	0	Over 13
45	62	W.A.	June	140	Nil for 3 days	5,000	0	—	43	5,270	—	17	0	—
46	26	W.A.	August	42	35 grains in a week	3,000	24	—	88	13,050	—	4	0	13
47	17	W.A.	August	35	Nil	2,000	8	—	69	7,610	—	11	9	15
48	18	W.A.	July	70	?	1,800	32	10th	64	5,640	67·5	19·5	0	Over 6
49	68	Rangoon	Sept. ?	49 ?	Little	?	4,450	—	83	16,170	45	?	0	Over 23
50	45	Bombay	October	65 ?	Some irregularly	?	7,688	—	—	5,610	44	?	10 grains daily	Over 20
51	38	W.A.	?	?	?	?	200	—	40	—	—	—	—	—

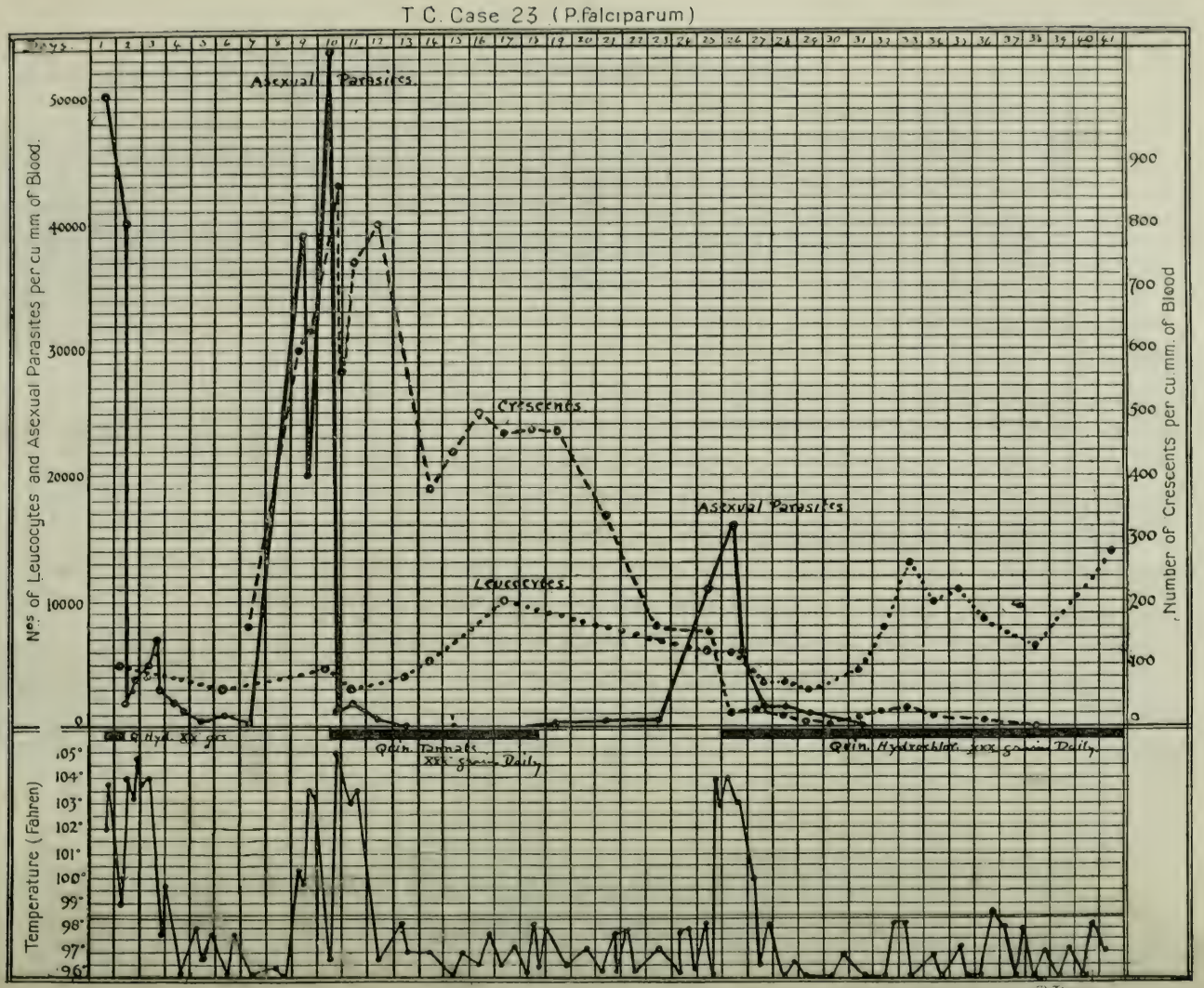
CASE 17.—R.B. *P. falciparum.*

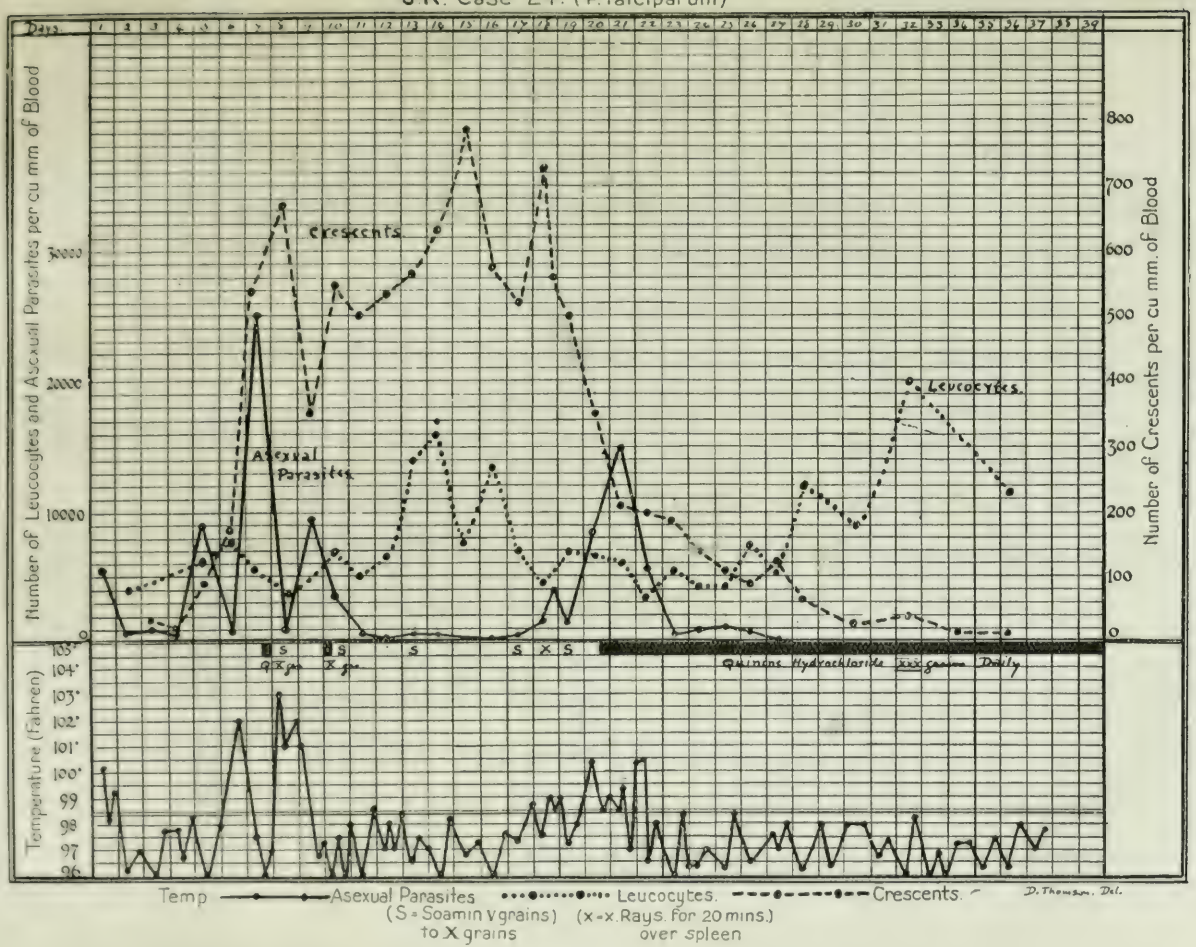



A.C. Case 18.(P.falciparum.)
Days
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
Nº of Asexual Parasites per cu.mm of Blood
2000
1000
0
Asexual Parasites.
Crescents.
Nº of Crescents per cu mm of Blood
400
300
200
100
0
Quinine Hydrochlor. grs V Daily
Qu'n. Tannat. xxx grs Daily
Qu. Hydrochlor. xxx grs Daily
Temp. (Fahren)
104°
103°
102°
101°
100°
99°
98°
97°
96°
D. Thomson Del.

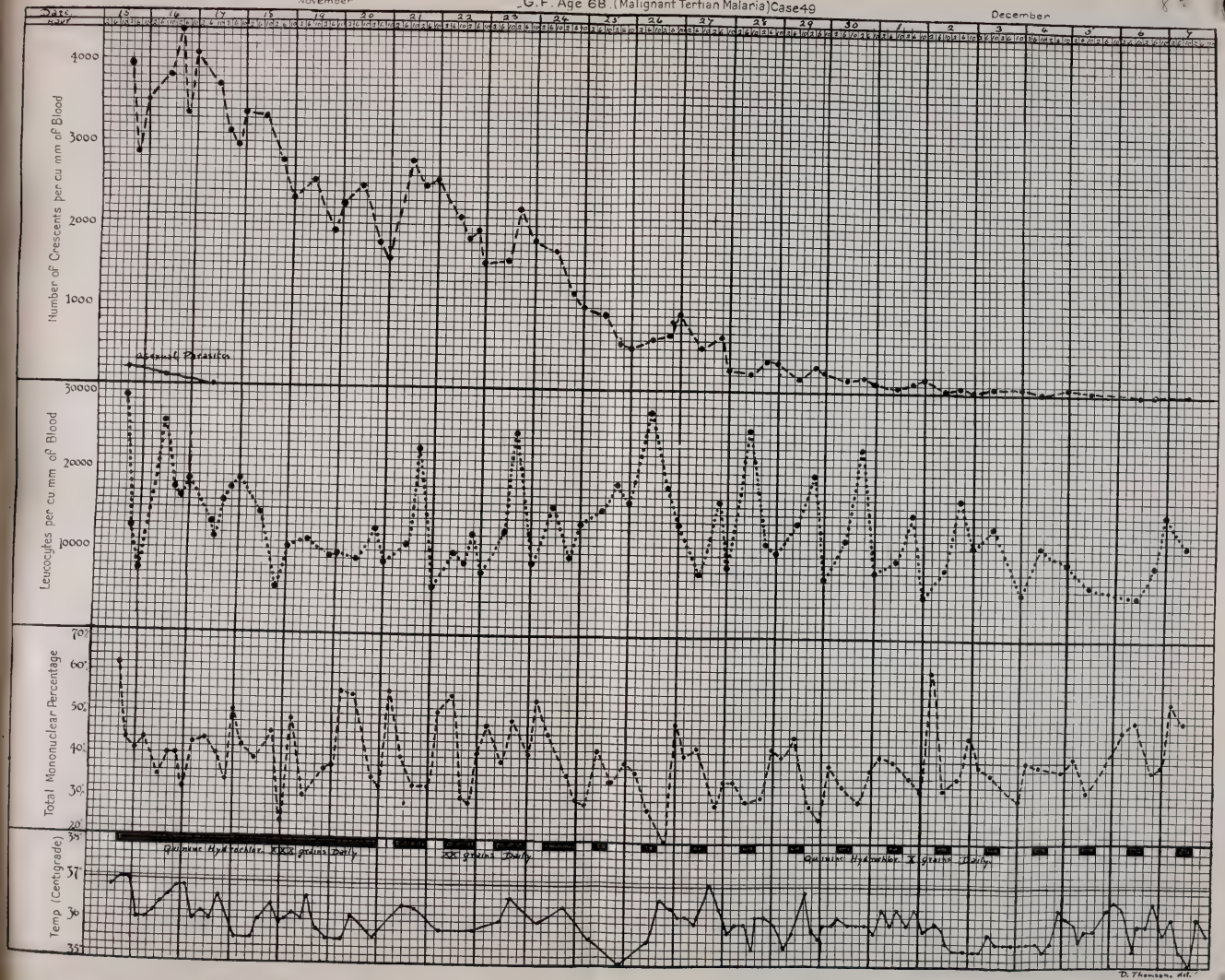
CASE 20.—F.B. *P falciparum.*




T C. Case 23 (P.falciparum)
Asexual Parasites.
Crescents.
Leucocytes.
Asexual Parasites
Nos of Leucocytes and Asexual Parasites per cu mm of Blood.
Number of Crescents per cu mm. of Blood
Temperature (Fahren)
Qu. Hyd. XX grs
Quin. Tannate XXX grains Daily
Quin. Hydrochlor. xxx grains Daily


J.K. Case 24. (P. falciparum)
Days
Number of Leucocytes and Asexual Parasites per cu mm of Blood
Number of Crescents per cu mm. of Blood
Crescents
Asexual Parasites
Leucocytes
Temperature (Fahren)
Quinine Hydrochloride xxx grains Daily
Temp
Asexual Parasites
Leucocytes.
Crescents.
(S = Soamin v grains) to X grains
(x-x. Rays. for 20 mins.) over spleen
D. Thomson. Del.


C H. (Malig Tertian Double) Age 25 Case 38
(P. Thomson Del.)
October
(November)
Numbers of Crescents per cu mm of Blood
Crescents
Numbers of Leucocytes & Asexual Parasites per cu mm of Blood
Mononuclear Percentage
Small Rings
Asexual Parasites
Total Leucocytes
Total Mononuclear
Quinine Hydrochloride gr xxx Daily
Patient had 4 days of Fever viz: on the 18th 19th 20th & 21st October
Temperature (Centigrade)
Crescents — Total Mononuclear Percentage.
Total Leucocytes
Total Mononuclear Leucocytes
Asexual Parasites — Temperature.


G.F. Age 68. (Malignant Tertian Malaria) Case 49
November
December
Date
Hour
Number of Crescents per cu mm of Blood
4000
3000
2000
1000
Asexual Parasites
Leucocytes per cu mm of Blood
30000
20000
10000
Total Mononuclear Percentage
70
60
50
40
30
20
Temp (Centigrade)
38
37
36
35
Quinine Hydrochlor. 10 gr. 4 hourly
10 grains Daily
Quinine Hydrochlor. 5 grains Daily
D. Thomson. del.

II.—THE LEUCOCYTES IN MALARIAL FEVER: A METHOD OF DIAGNOSING MALARIA LONG AFTER IT IS APPARENTLY CURED

BY

DAVID THOMSON, M.B., CH.B. (EDIN.), D.P.H. (CAMB.)

(*Received for publication* 8 *February*, 1911)

PREFATORY NOTE.

This research was carried on in the Tropical Ward of the Royal Southern Hospital, Liverpool, under the direction of Major R. Ross, C.B., F.R.S. The new facts obtained are the result of numerous daily observations extending over periods of several weeks. The laboriousness of the work was much diminished by the use of a new blood-counting pipette devised by the author. The method is based on 'The Thick Film Process' [Ross, 1903]. The funds for the research have been supplied by the Advisory Committee of the Colonial Office.

I. THE LEUCOCYTES DURING ACTIVE MALARIA *

During active malaria the number of leucocytes is, as a general rule, less than normal, but the numbers vary with the rise and fall of temperature. When the fever is in full force, that is during the rigor and high temperature, then there is a coincident diminution of the total leucocytes in the peripheral blood. In this diminution the polymorphonuclear as well as mononuclear leucocytes take part. If we take 7,000 per c.mm. as the normal leucocyte count, and the normal percentage of polymorphonuclears and total mononuclears as 65 per cent. and 35 per cent. respectively, then normally the polymorphonuclear leucocytes should number

* In this article I refer to charts in Paper I ('The Production, Life and Death of Crescents,' etc.).

about 4,550 per c.mm. of blood and the total mononuclears about 2,450 per c.mm. of blood. During a malarial temperature, however, the polymorphs are frequently less than 2,000 per c.mm., and the total mononuclears are also frequntly below this number. In some cases the total leucocyte count during the pyrexia may be as low as 2,000 per c.mm. of blood. In other exceptional cases, however, I have seen a marked leucocytosis during a high malarial temperature. This occurred in two cases of comatose malaria. It is rare, however, and I think it may be taken as a general rule that there is a leucopenia in malaria when the temperature is above normal. In other words, during the actual sporulation of the malarial parasites there is a diminution of the total leucocytes in the peripheral blood. This rule holds good with the benign tertian, malignant tertian and quartan parasites. Some observers have described a transient polymorphonuclear leucocytosis lasting only for one half-hour just at the commencement of a rigor. I have so far been unable to confirm this. After the sporulation is over and the temperature has fallen, it will be found that the total number of leucocytes has increased even up to normal numbers or more, so that in the intervals after the temperatures the leucopenia disappears, and may occasionally even have its place taken by a leucocytosis; but on the advent of the next sporulation and temperature, the numbers again fall. Thus with the rise and fall of temperature in malaria we have a corresponding fall and rise in the total number of leucocytes. The diminution of total leucocytes corresponds exactly with the rise of temperature, and the rise of total leucocytes corresponds exactly with the fall of temperature. See Charts N.B. Case 9, J.W. Case 30, and Billet's Chart [1908]. We may therefore enunciate a general law as follows:—

Law I. *During active malaria the number of total leucocytes in the peripheral blood is below normal, and varies more or less inversely with the temperature.*

When one analyses the different varieties of leucocytes, it is found that the mononuclears, and especially the large mononuclears, play most part in the variation enunciated in this law. In this research, however, I have considered only the total polymorphonuclear leucocytes and the total mononuclears. So many

transitional forms of leucocytes occur between the recognised varieties that a considerable personal factor comes into play, and in dealing with all varieties one increases the complications very much. Thus if we consider only the two chief classes of leucocytes with regard to Law I, it may be stated that the total polymorphonuclear leucocytes vary only slightly with the temperature in active malaria, but that the total mononuclears vary very much and inversely with the temperature. In other words, the sporulations of the malarial parasite cause a greater disturbance or reaction among the mononuclear than among the polymorphonuclear leucocytes. When the spores of the malarial parasite escape free into the blood plasma there occurs a mononuclear leucopenia, followed later by a mononuclear excess. This is exactly the reverse of what happens when a bacterial culture is injected intravenously into an animal. Here the result is a polymorphonuclear leucopenia followed by a polymorphonuclear excess (F. W. Andrewes [1910]).

In malarial fever the mononuclear leucocytes, especially the large variety, are undoubtedly the soldiers for defence. They ingest the spores of the malarial parasite in large numbers. Some hours after a malarial paroxysm or sporulation the large mononuclear leucocytes are found in great numbers filled with vacuoles and pigment, due to the active ingestion of parasites. If it were not for this phagocytic power of this class of leucocyte, it is likely that the parasites would become more and more numerous after each paroxysm, and the patient would soon succumb. When the mononuclears gain the upper hand and exist in large numbers the fever disappears spontaneously for a time. This is noticeable in Chart J.W., Case 30, where the patient had no relapse, though no quinine was given for fourteen days. This natural mononuclear defence is greatly assisted by the administration of quinine. After a dose of twenty to thirty grains of quinine the fever frequently disappears quite suddenly. Corresponding to this sudden disappearance of fever there is a fall in the number of parasites and a large increase of mononuclear leucocytes. This phenomenon occurs in many of the cases studied. The mononuclear increase continues so long as the fever does not return. It would therefore appear that an increase in the total mononuclear leucocytes is

coincident with periods of resistance or immunity to the disease. Previous to a relapse the number of leucocytes invariably falls (*vide* Charts W.M. [*P. vivax*], Case 17, R.B., and Case 20, F.B.). From these facts we have therefore the basis of another law, though this law is really only a different expression of Law I.

Law II. *In malarial fever the curve representing the percentage of total mononuclear leucocytes is the exact inverse of the temperature curve*: in other words, if one makes frequent daily differential counts of the leucocytes in malarial fever, on charting the varying percentage of total mononuclears, one finds the curve obtained is the exact inverse of the temperature curve. See Charts M.G. (*P. vivax*), and G.J., Case 34 (*P. falciparum*).

When the temperature in malaria is rising, the total mononuclear percentage is falling, and *vice versa* the fall of temperature is exactly simultaneous with a rise in the total mononuclear percentage. At the height of his fever, J.W., Case 30, had only 39·4 per cent. of total mononuclear leucocytes. He received twenty grains of quinine. Next day he had no fever, and the total mononuclears were 80 per cent. This rise is chiefly due to the large mononucleated variety. In Case 8, P.D., after the first dose of quinine the fever soon ceased, and the large mononuclears rose from 19 per cent. to 65 per cent. in one day. I have observed occasionally a total mononuclear percentage as high as 90 per cent. I have not met with any exceptions so far to Law II. It would seem that a high mononuclear percentage is incompatible with a true malarial temperature.

From the above law it would seem reasonable, as a therapeutic measure in malaria, to attempt to increase the leucocytes, especially those of the mononuclear type. Recently it was discovered that an injection of an extract of leucocytes, causes a marked increase of leucocytes in the peripheral blood in a few hours (Ross and Thomson [1911], Alexander [1911]). A case of malaria was treated with this substance (Lambert [1909]), and it was found that the injection prevented the rigors from occurring. It is highly probable that this phenomenon was due to the increase in leucocytes following these injections. The extracts made and used by Alexander consist chiefly of the polymorph variety of leucocytes, and produce, after injection, chiefly a polymorph excess. We would

expect to obtain better results from injections of a mononuclear leucocytic extract, but unfortunately so far, it is very difficult to obtain such a substance. Before leaving the question of therapeutics, I would refer to Charts J.M. (leprosy), D.T. (normal person), and J.L., Case 53 (*P. vivax*). From these it will be seen that injections of pilocarpine in doses of one-tenth of a grain produce quite a definite excess of the mononucleated variety of leucocytes in the peripheral blood, (*vide* researches by Waldstein [1895]). In Chart J.L., Case 53, it will be noticed that during the injections of pilocarpine in the above doses the mononuclear percentage as well as the total leucocytes maintained a high level, and that the fever disappeared without other treatment. As this, however, frequently occurs naturally without any treatment when patients are kept in bed in comfortable circumstances, one cannot come to definite conclusions until further work has been done in this line.

II. THE BEHAVIOUR OF THE LEUCOCYTES IN QUIESCENT OR LATENT MALARIA, AND IN MALARIA APPARENTLY CURED BY QUININE OR OTHER TREATMENT

Laws I and II deal with the leucocytes where the malarial parasites are numerous and easily found and where true paroxysms of fever occur. When, however, we observe further the leucocytes during the latency or apparent convalescence and cure of the disease, we find some remarkable differences. During active malaria, as already stated, the leucocytes, on the whole below normal numbers, show periodic variations in number, due chiefly to fluctuations of the mononuclear variety. In convalescent or apparently cured malaria, however, where the temperature remains more or less below normal, we have a similar periodic variation; but this time the variations are due chiefly to fluctuations in the polymorphonuclear variety, and the leucocytes on the average are greatly in excess of the normal number (*vide* Chart C.H., Case 38). This change from a fluctuation in the number of mononuclears to a greater fluctuation in the number of polymorphonuclears seems to take place invariably. A certain time elapses after the active malaria before this new leucocytic phenomenon begins. If the malaria has been severe, or if the patient is much debilitated, it is

not noticeable for perhaps ten days or longer, while in other cases, usually less severe, it may occur in a few days to about a week. It also occurs whether the patient has recovered as a result of quinine treatment or not, and is therefore not due to quinine treatment (*vide* Chart W.M. (*P. vivax*)). Again the fluctuation is very markedly periodic, occurring daily or on alternate days or irregularly. Thus if the fever was quotidian, tertian or irregular there is evidence to show that these fluctuations in total leucocytes (chiefly due to polymorphs) are also quotidian, tertian or irregular. This would seem to indicate that there is some malarial periodic virus lingering in the system in spite of vigorous quinine treatment of thirty grains daily, and where no fever parasites can be detected on microscopic examination of the blood. If the total mononuclear percentage is charted as before, it is found that the percentage is low when the total leucocytes are high, and the curve representing the total mononuclears does not vary nearly so much as the curve of the total polymorphonuclears (*vide* Chart 38, C.H.). Law II, however, still holds good, the mononuclear percentage being as a rule lowest when the temperature is highest, although the temperature may at no time rise above normal. It would thus appear that this transient periodic increase of leucocytes, chiefly due to polymorphonuclears, is due possibly to minute numbers of asexual parasites sporulating at regular intervals. If this is so, then it would appear that the sudden liberation into the blood of large doses of the malarial virus causes a leucopenia, while small doses cause a leucocytosis. This periodic leucocytosis occurs at the time of the day at which the patient previously had a rigor and fever. Another possible explanation is that it is due to the periodic outburst of sexual forms or gametes into the peripheral circulation. (See accompanying paper on ' Crescents.') Against this explanation, it is found to occur when no gametes have been produced, and also long after they have disappeared owing to quinine treatment. In Case C.H. 38, it almost seems as if the daily outburst of crescents caused a daily polymorphonuclear increase. It would seem, however, that the polymorphonuclear leucocytes seldom or never ingest crescents. In cases with very numerous crescents and absence of asexual parasites many pigmented mononuclear leucocytes are found, but only very occasionally does one find a

pigmented polymorphonuclear leucocyte. It would therefore appear that the mononuclear leucocytes ingest not only the asexual parasites, but also the sexual forms or gametes. Whether the gametes can be ingested alive or only after their death is a matter for speculation. It is interesting to note that the change from a mononuclear into a polymorphonuclear swing often takes place about the time that crescents first appear in the peripheral circulation, that is a week to ten days after the last paroxysm of fever. In the preceding article on 'Crescents,' pages 65 and 66, I have pointed out that an increase of leucocytes seems to be coincident with increase of immunity and consequent crescent production, and that in quiescent malaria the leucocytes are in greater numbers than normal. These periods of quiescence often continue for ten or more days, though no quinine be given, and it would almost seem that the relapses tend to occur when the polymorphonuclear leucocytes begin to play more part in the increase of leucocytes. This, however, is only speculation. Beyond these suggestions, I am unfortunately unable to give any explanation of this most interesting phenomenon, which I will enunciate as Law III.

Law III. *In convalescent or apparently cured malaria, transient periodic leucocytoses occur in the peripheral blood, and these leucocyte fluctuations arise chiefly from a polynuclear variation.*

In these periodic fluctuations the leucocytes often reach numbers as great as 40,000 to 50,000 per c.mm. of blood, and the mononuclear percentage may rarely fall as low as 20 per cent. of the total leucocytes. On one occasion I observed a leucocytosis of 125,000 per c.mm. Two hours later the number had fallen to 22,000 per c.mm., and eight hours later to 6,000 per c.mm. (Chart T.H. (*P. falc.*)).

III. A METHOD OF DIAGNOSING MALARIA NOT ONLY DURING THE ACTIVE STAGE BUT ALSO IN LATENT CASES, AND IN CASES APPARENTLY CURED BY CONTINUED QUININE TREATMENT

This new method of diagnosis depends on the constancy of Laws II and III. As already stated, there is a periodic fluctuation in the percentage of total mononuclear leucocytes in malaria. In active malaria this is due mainly to a variation in the number of

mononuclear leucocytes. This periodic fluctuating mononuclear percentage still continues in latent malaria and in cases thoroughly treated with quinine, but in these latter it is due mainly to a variation in the number of polymorphonuclear leucocytes. To apply this diagnostic test one must take frequent smears of the suspected person's blood; one smear every four to six hours is sufficient. These should be taken during a period of two to three days. Make differential counts estimating the total mononuclear percentage in each smear and plot the curve representing these percentages. If there is a more or less periodic variation in the percentage amounting to over 20 per cent., then the patient has, or has had, malarial fever. In active malaria the total mononuclear percentage usually varies somewhere between 20 per cent. and 80 per cent., the periodic swing having usually an amplitude over 20 per cent. and often as high as 40 per cent. and more. In latent and apparently cured malaria the mononuclear swing occurs at a lower level somewhere between 20 per cent. and 60 per cent. The amplitude as before is usually about 20 per cent. or over.

If the swing is irregular, then it denotes that the patient has, or has had, malarial fever of an irregular type. If the swing is distinctly quotidian or tertian, it denotes that the patient has, or had, quotidian or tertian paroxysms of fever. On examining the blood of a normal person in this way, I find that the swing of the total mononuclear percentage does not amount as a rule to more than 10 per cent., if one counts from 150 to 200 leucocytes. This 10 per cent. swing is quite irregular, and is due mainly to the error arising from the estimation of an insufficient number of leucocytes. Slight changes do, of course, take place in normal persons after exercise, meals, etc., but Chart D.T. (normal), shows clearly that these natural variations are much smaller than the marked oscillations which take place in malaria cases (*vide* Chart D.O., Case 52 (*P. vivax*), and others). Again in certain septic diseases one may have a swing in the total mononuclear percentage, but in such cases the percentage falls to a very low limit, as low as 8 per cent. to 10 per cent., and will seldom rise as high as 30 per cent., whereas in malaria the highest point of the mononuclear percentage will seldom be lower than about 45 per cent. to 50 per cent. Thus I am led to believe that a large periodic variation in the total mononuclear percentage amounting to 20 per cent., and

where the upper limit reaches over 45 per cent., is pathognomonic of malaria, and is furthermore so delicate a test that the presence of the disease or its dregs can be detected long after continued quinine administration. Moreover, by careful estimation of the percentage in this way one can also state whether the previous active fever was quotidian, tertian, or irregular in nature. It is also of great value to estimate several times daily the total leucocyte count; because, as already stated above, remarkable periodic variations in number take place, especially in quiescent or latent cases. Most of the cases show a quotidian rise of leucocytes (*vide* Charts C.H., Case 38, T.H. (*P. falc.*), etc.). The height of the leucocyte rise corresponds exactly with the lowest point of the total mononuclear percentage. This shows that this rise of leucocytes is not a digestion leucocytosis. The increase in the number of leucocytes after a meal is principally a lymphocyte increase, also the variation in number is too great to be accounted for by the natural increase after a meal. In normal persons the leucocytes seldom reach 15,000 per c.mm. after a meal. When they reach numbers over 15,000 per c.mm. it denotes some pathological condition (Beattie and Dickson [1908]). In malaria the height of the periodic rise is often as great as 30,000 per c.mm. of blood, and more rarely reaches a number as high as 40,000 to 50,000 per c.mm. Chart D.T. (normal person) shows that the natural daily variation seldom reaches 14,000 to 15,000 per c.mm. of blood.

One naturally wonders how long this abnormal leucocytic phenomenon continues after the last active attack of malarial fever. I am unable so far to answer this question, but can give some remarkable instances which show that it may continue for months, and possibly even for years.

(*a*) Case 38, C.H., shows the phenomenon with unabated force after three weeks' continuous administration of quinine 30 grains daily. Case D.O., 52, shows the same after one month of continuous treatment with quinine twenty grains daily and methylene blue twelve grains daily.

(*b*) Case J.M. (leprosy) showed the same phenomenon. This was noticed while examining the blood to see if there was any leucocyte change taking place which might be characteristic of leprosy. To my surprise the mononuclear percentage curve indicated a previous quotidian malaria. On enquiring into the

past history of this case, it was found that he had suffered from malaria four years previously. He had had no recurrence of fever since then, but stated that he remembered having a slight shivering attack five months previous to this blood examination. So far as I am aware, no one has ever described a mononuclear leucocyte excess as a characteristic of leprosy, so that one is led to the conclusion that the high mononuclear percentage with large variation was due to an infection of malaria long since apparently cured.

In the above method of diagnosis it is important to remember the already well-known fact that there is an excess of large mononuclear leucocytes in malaria. Stephens and Christophers [1908] consider a value of this variety of leucocyte above 15 per cent. as diagnostic of malaria, and there is no doubt but that these large mononuclears play most part in the total mononuclear fluctuation occurring in active malaria. They also occur in numbers above normal in apparently cured cases of malaria. Thus a diagnosis of malaria might be made from one single differential leucocyte count where the total mononuclear percentage is high, say above 50 per cent., and especially where the large mononucleated variety is in excess. Normally the large mononuclears vary from 1 per cent. to 3 per cent. of the total leucocytes (Beattie and Dickson [1909]).

The two following cases show that malaria may be diagnosed from one single differential count even months after an apparent cure:—

(*a*) Case I, W.M. (mixed infection), left hospital in March, 1910, after a thorough course of quinine and methylene blue treatment. On his discharge no parasites could be detected in his blood. He continued to take quinine in doses of five to ten grains daily until July, 1910, when he returned to hospital to report himself. During this time he had not left England. Examination of his blood revealed no parasites, but showed a total mononuclear percentage of 67 per cent. His blood was therefore still abnormal more than five months after his last attack of fever. The patient was at this time quite well.

(*b*) Case A.L.B. was treated in hospital during June and July, 1910, for malignant tertian malaria followed by blackwater fever. He returned to hospital six months later in February, 1911, to

report himself. He stated that he had been quite well for several months. His blood nevertheless showed that the total mononuclear leucocytes amounted to 62 per cent. No parasites could be detected.

Though malaria may therefore be diagnosed, or at least suspected long after its apparent cure, from one differential count, yet one count is not sufficient as a rule in these cases. The blood might happen to be taken at the lowest limit of the mononuclear swing, and might reveal a total mononuclear percentage of, say, only 20 per cent. Thus one estimation only might give a negative result, whereas several differential counts taken six hourly for two to three days would give a positive result. I think sufficient has been said to show that malaria, or at least its dregs, remain in the system for a long time after an apparent cure. It would almost seem certain that the parasites remain in the host for very long periods in spite of vigorous quinine treatment, and that they continue to develop and sporulate, giving rise to the periodic leucocyte variations described above. The case of P. T. Manson is well known, in which a relapse occurred nine months after the original infection followed by three months of quinine treatment, and where re-infection was impossible.

Before concluding, I should state that the leucocytic phenomena in blackwater fever show the malarial origin of that disease. In blackwater fever, as in malaria, the mononuclear percentage would seem also to vary inversely with the temperature.

SUMMARY

(1) During active malaria the number of leucocytes in the peripheral blood is decreased. During quiescent malaria, and in cases apparently cured by treatment, the leucocytes in the peripheral blood are much increased.

(2) During the rigor and temperature in malaria, the mononuclear leucocyte percentage (more especially that of the large mononucleated variety) is low. With the fall of temperature, however, the mononuclear percentage rises very high, sometimes even to 90 per cent. of the total leucocytes. This fluctuation in the percentage of total mononuclear leucocytes occurs also long after

continuous quinine treatment, and is observed for months and even years (?) after the last attack of fever.

(3) In these apparently cured cases of malaria, the mononuclear percentage is lowest at the time of the day at which the rigor and fever occurred during the previous active malaria; and, moreover, at this time there also occurs a very marked leucocytosis, which continues only for a few hours. The leucocytes often reach numbers as great as 30,000 to 50,000 per c.mm. of blood. On one occasion they were as numerous as 125,000 per c.mm. Two hours later they had fallen to 22,000 per c.mm., and in eight more hours there were only 6,000 per c.mm. This case showed a regular daily periodic variation in the number of leucocytes, averaging from about 6,000 per c.mm. to 50,000 per c.mm. The height of the rise always occurred about noon. This was the time at which the rigor and fever, which was quotidian, was wont to occur previously. This post-malarial leucocyte phenomenon occurred always without exception in the forty cases examined, and would therefore seem to be an infallible sign of previous malaria, as, so far, it has not been observed in any other disease.

(4) It would appear that large numbers of malarial parasites on sporulating cause a leucopenia, while a very small number on sporulating cause a leucocytosis.

REFERENCES

Alexander (1911), 'The Use of Leucocytic Extract in Infective Processes.' Brit. Med. Journ., Feb. 18.

Alexander, Nauss, and Williams (1911), 'The Use of Leucocytic Extracts in Infective Processes.' Liverpool Med. Chir. Journ., Jan.

Andrewes (1910), Croonian Lectures, Lancet, July.

Beattie and Dickson (1909), Special Pathology, p. 88.

Billet, A. (1901), 'De la Formule Hémo-leucocytaire.' Bulletin Médical de l'Algérie.

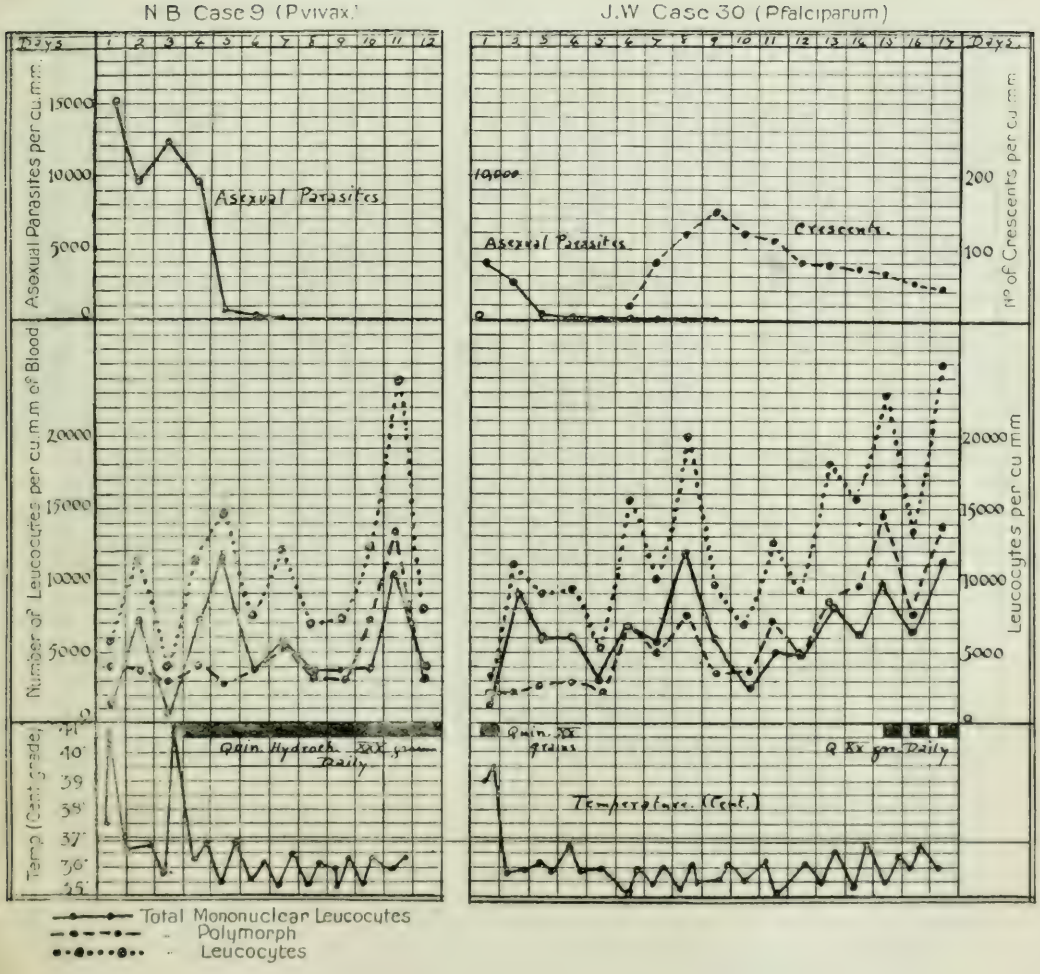
Lambert (1909), Amer. Journ. Med. Sciences, Vol. CXXXVII, p. 506.

Ross (1903), 'The Thick Film Process for the Detection of Organisms in the Blood.' Thompson Yates and Johnston Laboratories' Report, Vol. V, Part 1.

Ross and Thomson (1911), 'A case of Sleeping Sickness Studied by Precise Enumerative Methods: Further Observations,' Proc. Royal Soc., B, Vol. LXXXIII.

Stephens and Christophers (1908), Practical Study of Malaria and other Blood Parasites, p. 221.

Waldstein (1895), Berliner klinische Wochenschrift, April 29 and May 6.


N B Case 9 (P.vivax)
J.W Case 30 (Pfalciparum)
Days
Asexual Parasites per cu.mm.
Asexual Parasites
Number of Leucocytes per cu.mm of Blood
Qnin. Hydroch. XXX grains Daily
Temp (Cent. grade)
Crescents
Nº of Crescents per cu.mm.
Leucocytes per cu.mm
Qnin. XX grains
Q. Xx gn Daily
Temperature (Cent.)
Total Mononuclear Leucocytes
Polymorph Leucocytes

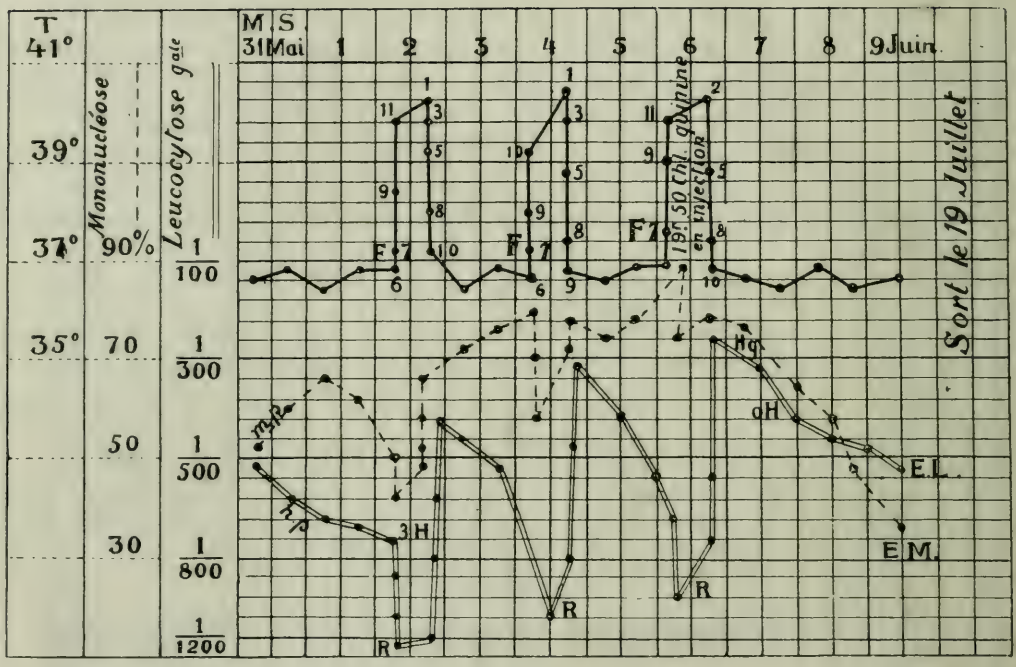
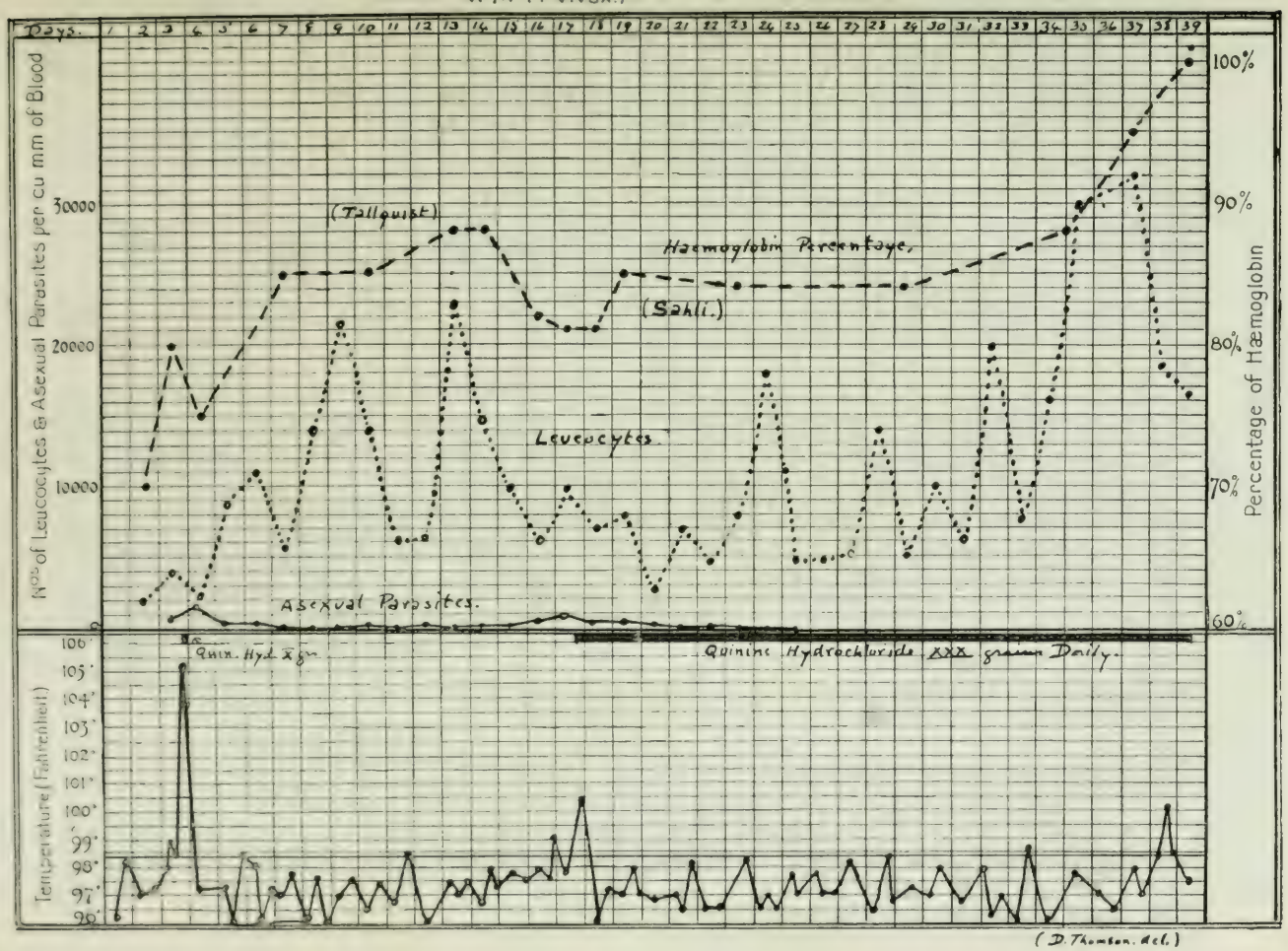
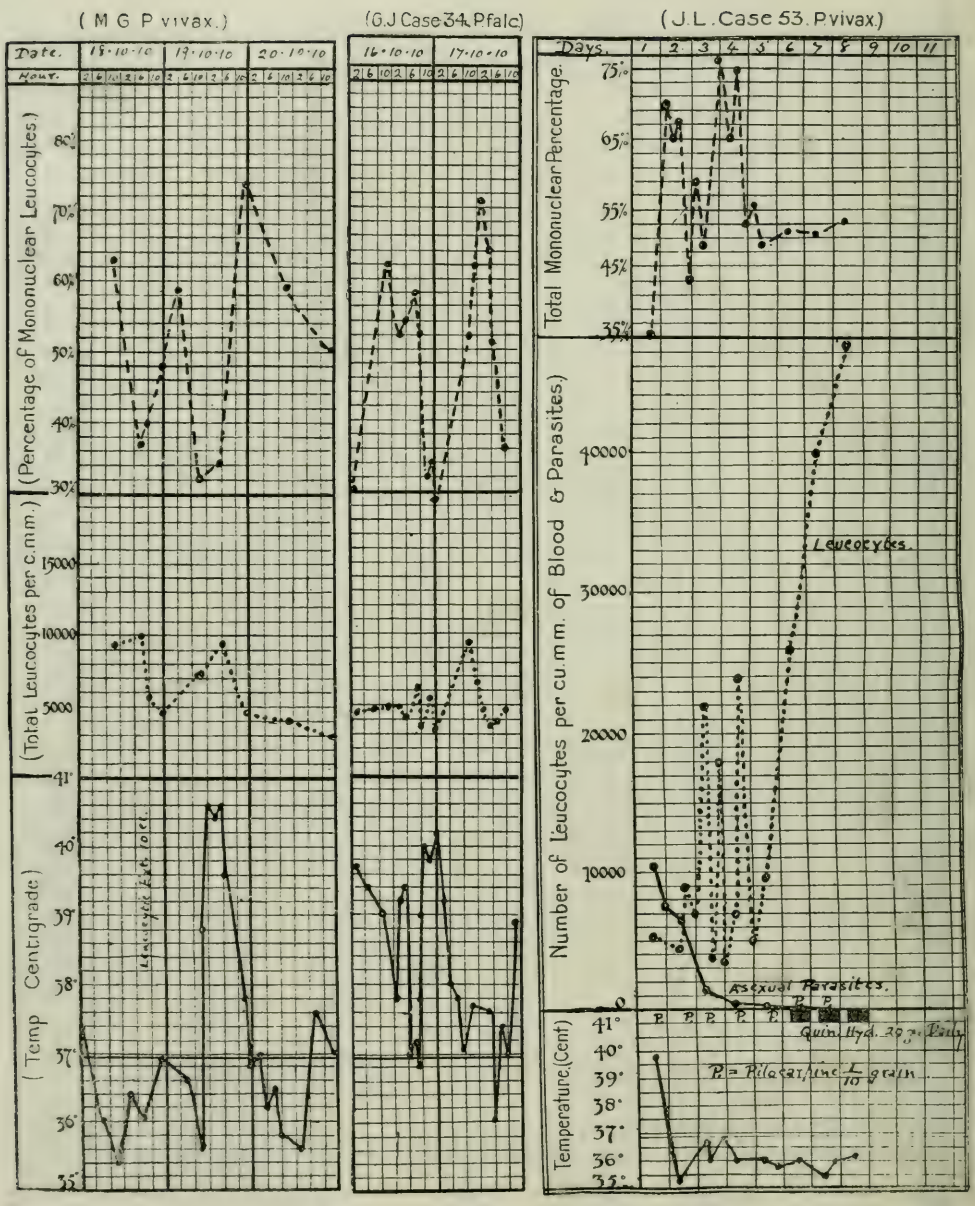
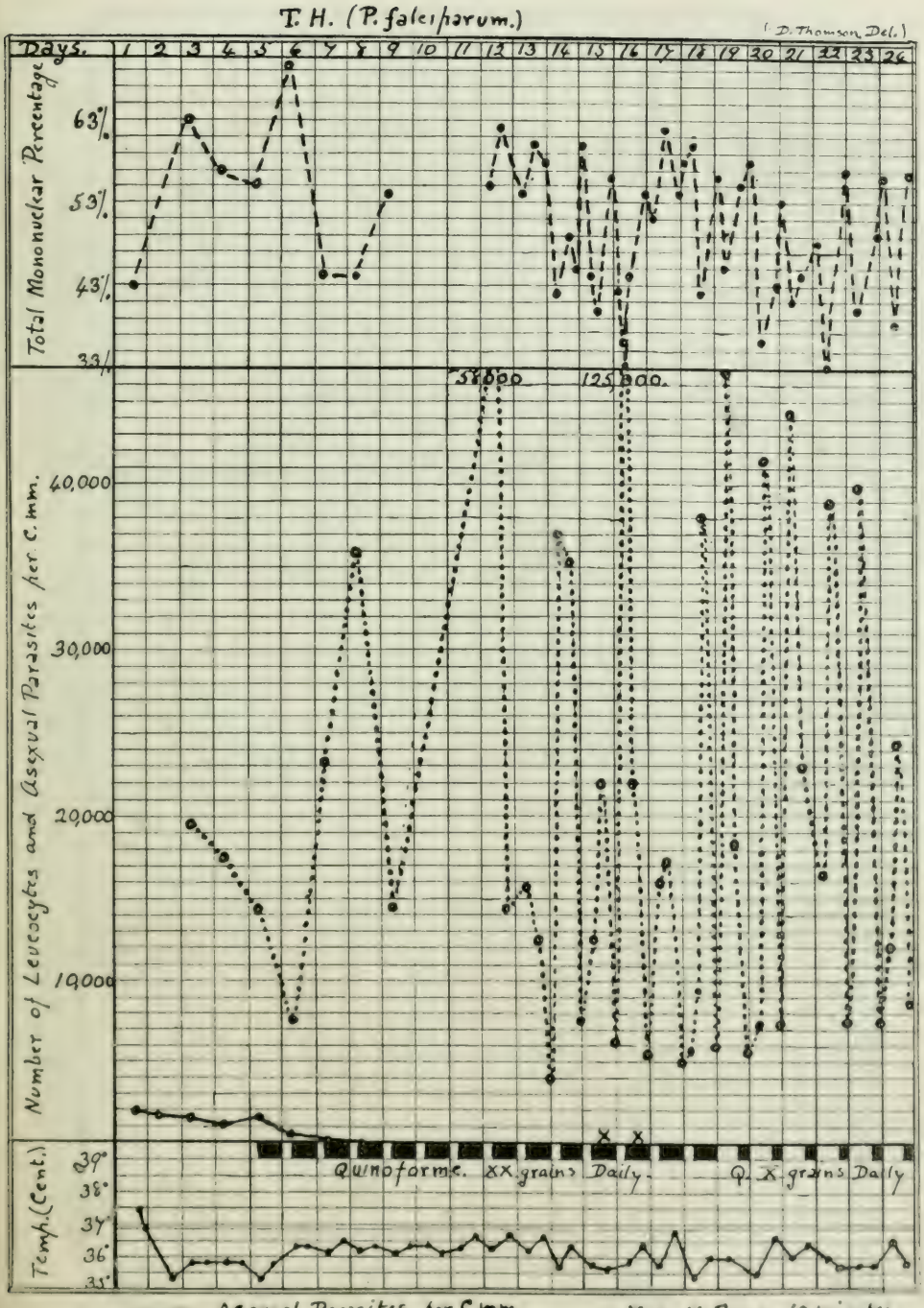


Chart shewing the changes in the total Leucocytes (and in the percentage of mononuclears large and small) in a case of simple tertian fever. The double line curve = that of total Leucocytes. (*After* Billet)


W M (P vivax.)
Days.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39
Nos of Leucocytes & Asexual Parasites per cu mm of Blood
30000
20000
10000
0
100%
90%
80%
70%
60%
Percentage of Hæmoglobin
(Tallquist)
Haemoglobin Percentage.
(Sahli.)
Leucocytes.
Asexual Parasites.
Quin. Hyd. X gr.
Quinine Hydrochloride XXX grains Daily.
Temperature (Fahrenheit)
106°
105°
104°
103°
102°
101°
100°
99°
98°
97°
96°
(D. Thomson. del.)

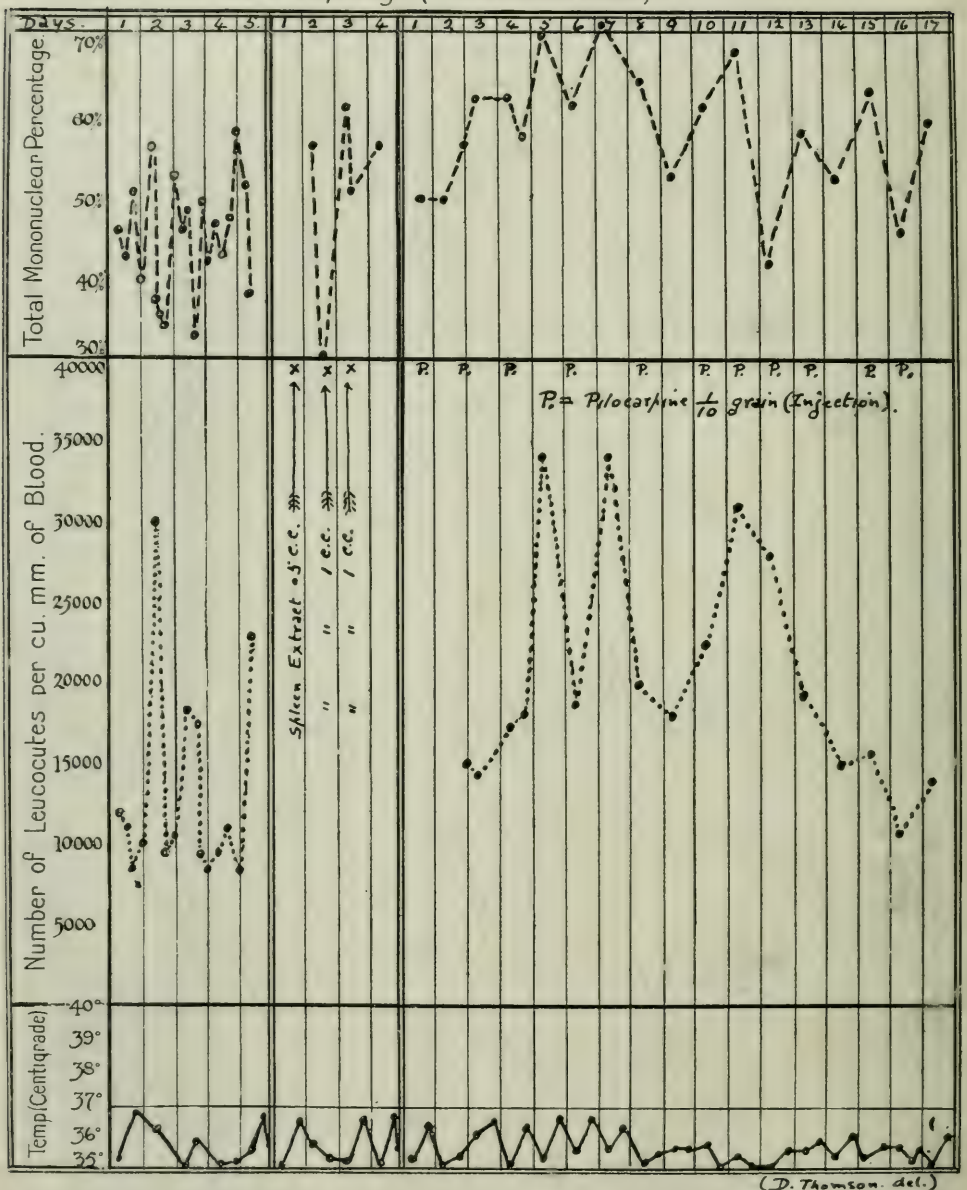

(M G P vivax.)
(G.J Case 34. Pfalc)
(J.L. Case 53. P vivax)
Date.
Hour.
18·10·10
19·10·10
20·10·10
16·10·10
17·10·10
Days.
(Percentage of Mononuclear Leucocytes.)
(Total Leucocytes per c.mm.)
(Temp Centigrade)
Total Mononuclear Percentage.
Number of Leucocytes per cu.mm. of Blood & Parasites.
Leucocytes.
Asexual Parasites.
Quin. Hyd. 20 gr. Daily
P = Pilocarpine 1/10 grain
Temperature (Cent)


T. H. (P. falciparum.)
(D. Thomson, Del.)
Days.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Total Mononuclear Percentage
63%
53%
43%
33%
Number of Leucocytes and Asexual Parasites per C. mm.
40,000
30,000
20,000
10,000
38,000
125,000
Temp. (Cent.)
39°
38°
37°
36°
35°
Quinoforme. XX grains Daily.
Q. X. grains Daily
Asexual Parasites per C. mm.
Leucocytes per C. mm.
Total Mononuclear Percentage.
X = X Rays 10 minutes over Spleen.

D.T. (Normal)
Number of Leucocytes per cu. m.m. of Blood.
Total Mononuclear Percentage.
Days
Leucocytic Extract 1/3mm emigrated.
Spleen Extract 1.c.
x Pilocarpin 1/10 grain
x Pilocarpin 1/4 grain

D.O. Case 52. (P.vivax.)
Days
Total Mononuclear Percentage
No. of leucocytes & Asexual Parasites per cu.mm
Temperature (Cent.)
Methylene Blue xii grains Daily.
Quinine Hydrochlorid xx grains Daily
Asexual Parasites.
Leucocytes.
No Parasites (Search for 4 hours)
Leucocytosis
Total Leucocytes.
Start Rigor.

J.M. Leprosy. (Old Malaria Case)



NOTE UPON YELLOW FEVER IN THE BLACK RACE AND ITS BEARING UPON THE QUESTION OF THE ENDEMICITY OF YELLOW FEVER IN WEST AFRICA

BY

SIR RUBERT BOYCE, F.R.S., M.B.

(*Received for publication* 25 *February*, 1911)

The question of the susceptibility of the various races of mankind to yellow fever is one which has always attracted considerable attention, and one about which there still exists very great misunderstanding.

The medical authorities on yellow fever in the 18th and 19th centuries held that no races were absolutely immune, with the possible exception of the Chinese. They held strongly, however, to the view that the disease was very rare amongst the native inhabitants of tropical countries, whilst very common on the other hand amongst new arrivals. In other words, the older authorities stated that yellow fever was essentially an acclimatising fever.

Inasmuch as the Latin races were the first to colonise, they were the first to suffer from yellow fever. Thus we have very complete records of continual epidemics amongst the Spaniards, Portuguese and French. So frequent, in fact, were these epidemics that the Latin races were considered more susceptible than the other races.

When, however, the northern races began to colonise, yellow fever proved itself equally virulent amongst them as the records of Dutch, Danish, English, Norwegian and Swedish colonisation and immigration amply testify.

It thus came about that yellow fever was a measure of commercial and maritime expansion, and of labour movements in the various industrial centres in the tropical world.

A period then arrived when, as in the 18th century, medical men observed that the offspring of whites who were born in yellow fever districts later escaped the disease during epidemics of yellow

fever. Then it also became apparent that the size of the epidemics was strictly proportional to the number of new arrivals.

This observation brought out into still greater prominence the fact that the permanent inhabitants of tropical towns did not die in the same proportion as the new comers. These facts were observed over and over again in New Orleans, in the West Indies, in Central and in South America, the most careful tables for comparison being furnished by Rio de Janeiro.

It was therefore concluded that the permanent inhabitants, whether Creoles as in the Southern States and in the West Indies, or Indian-Spanish as in Central and South America, were to a large degree immune, but that they lost this apparent immunity if they went to reside in Europe or a cold climate for a long period. The question why they were immune, however, only attracted scant attention, and at most, shrewd surmises were attempted like those of the great Faget of New Orleans. It was not, in fact, until the mosquito doctrine was firmly established that scientific attention was given to this exceedingly interesting fact.

In the slave trade period, when black labour was introduced into the West Indies, Central and South America, and into the Southern States of America a new series of facts became patent. Sometimes the blacks died in very large numbers from yellow fever, as in the Philadelphia epidemic, but in most cases where we are in possession of reliable figures like those furnished by Chassaignac, Roche, Lazard, Brady and numerous others, the proportion of deaths amongst the slaves or their descendants was relatively small. It was conclusively shown during the 1905 New Orleans epidemic of yellow fever that the blacks could get yellow fever. Thus Lazard states that in that epidemic there were 452 fatal cases amongst the whites and six amongst the negroes, whilst Chassaignac observed that the blacks were liable to the disease equally with the whites, but had it in a *particularly mild form*. Chassaignac's figures are as follows:—

In one series of the observations the mortality amongst ninety white cases was 20 per cent., and amongst 950 coloured cases 1·2 per cent. In another series of 500 cases amongst the whites there was a mortality of fifty-one, and amongst 200 coloured a mortality of one. La Roche states that the mortality from yellow fever in

Jamaica amongst the troops was 102 per thousand amongst the white soldiers, and eight per thousand amongst the blacks. Blair states that of the 1,790 black men imported into Demarara none died of yellow fever during the 1852 epidemic of that disease. The fact was therefore abundantly proved that whilst yellow fever did occur in the blacks, it nevertheless did not assume the same severe type as in the whites; fatal cases did, however, occur from time to time amongst the blacks, and in some epidemics there was a comparatively high sickness rate. It became evident that, therefore, the black possessed no natural race immunity, and that it was only a question as amongst the whites of 'acclimatisation,' that is, of coming from a district or country where yellow fever was rare into a city where yellow fever happened to be endemic.

The new comer, whether black or white, was liable to the disease. In this connection it is interesting to note that Coolies and Chinamen are also liable to yellow fever.

All these are facts which go to prove that the various races of mankind are susceptible to yellow fever, and that there is no absolute racial immunity.

The question has now proceeded a stage further owing to the increased attention paid to yellow fever in West Africa.

I have examined very closely the recorded outbreaks of yellow fever in West Africa, and it soon became abundantly clear that the so-called classical type of yellow fever was comparatively rare amongst the native races. In the various recorded epidemics the medical authorities of the time drew attention to the disproportion of the death-rate amongst blacks and whites. This fact was all the more remarkable as the natives far outnumbered the whites, and lived in notoriously over-crowded and insanitary conditions, and, as we now know, in an atmosphere crowded with the Stegomyia.

Why, therefore, if there was yellow fever on the coast of Africa, as was abundantly shown by the very numerous outbreaks amongst the whites, did no large epidemics occur amongst the black natives, and depopulate the West Coast? This is a very pertinent question and requires a very definite answer.

The West African blacks can get yellow fever, of this we have absolute proof, notably in the epidemics of yellow fever on the Coast in 1910. As far back as the epidemic of 1884 in Freetown,

a case of yellow fever was recorded in a native, and two cases amongst the black soldiers of the West Indian Regiment. In 1910, however, we have recent and positive evidence from the clinical histories and post mortem examinations. At Freetown, for example, one fatal case was recorded in a West Indian native soldier in July of last year, and also a fatal case in a native of Freetown. At Sekondi, in 1910, two cases amongst black men are recorded during the outbreak. In the two places there was a total of seventeen cases recorded amongst the white and five amongst the black residents. In October, 1910, according to Sorel, a small outbreak occurred at Grand Bassam, and three cases were recorded amongst natives. Therefore, it is beyond dispute that yellow fever can occur in its severe and fatal forms amongst the West African black races. This, then, corroborates the opinions of the older clinical observers that the black races were not absolutely immune. A new light, however, is thrown upon the problem by the 1909 epidemic of yellow fever in Barbados, which I was called upon to investigate. In this epidemic, yellow fever proved more fatal amongst the blacks than the whites. Out of a total of eighty-six cases, fifty-four occurred amongst the black inhabitants.

The blacks of Barbados are the descendants of the original imported African slaves; clearly, therefore, there was no hereditary racial immunity.

But why should the same race in West Africa appear to be immune? The answer to this question is the solution of the question of the presence of yellow fever in Africa. The Barbadian black lived in recent years under favourable conditions. The Stegomyia, there is every reason to believe, was greatly reduced in numbers by the introduction of a pipe-borne water supply laid on to the houses or to stand-pipes along the roads; puddles of water are not met with owing to the very porous nature of the soil, and the yards had been kept fairly free from odd water containers. There is practically no bush in the towns and villages, and the island is very much wind-swept. All these are factors which would tend to the diminution of the Stegomyia. The last recorded epidemic of yellow fever prior to the 1909 outbreak occurred in 1881, that is twenty-seven years previously. Therefore, it is reasonable to assume that yellow fever was not endemic on the island, and that

all those natives born since the 1881 epidemic were absolutely non-immunes. It is not surprising, therefore, that they became infected in those districts where the Stegomyia was present in sufficient numbers, and when the virus had been introduced into the island from without. The reverse is the case in West Africa.

The evidence, therefore, is conclusive—

1. That the negro can contract and die from yellow fever.

2. That he has, as a rule, yellow fever of a much milder type than that met with amongst whites who have recently arrived in a tropical country.

Naturally it follows that it is reasonable to ask: Does yellow fever occur amongst the natives of West Africa in a mild form, difficult of recognition; just as we know it did amongst the Creoles of the West Indies and the indigenous inhabitants of New Orleans, Cuba, Rio, Vera Cruz, Pará, etc.? In my opinion this is the only reasonable hypothesis which the facts will support and, moreover, it is one which has become formally adopted by those who have specially studied yellow fever, notably Marchoux and Simond, Otto and Neumann, Durham, and the American Cuban Commissions.

It is notorious that in places like Rio, Pará, and other endemic centres in the past, yellow fever was regarded as a disease of the foreigner or new-comer and not of the native; only the foreigners acquired the severe black vomit and died, the permanent inhabitants escaped. From the time of Faget, of New Orleans, up to the present date observers have, however, come to the conclusion that yellow fever does occur in the native children, and that it can occur more than once amongst adult natives. In other words, the natives suffer in early childhood and may suffer from subsequent attacks. We now can understand why fatal or severe yellow fever is rare amongst the native population—the natives are partially immunised. They contain the virus, nevertheless, in their blood, and can infect the Stegomyia. If, on the other hand, these same natives are removed in childhood from a yellow fever endemic area and protected, they become rapidly non-immunes, as shown above in the case of Barbados, and as has been proved many times amongst the Creoles and Indian-Spanish races.

The evidence is that yellow fever, like most other infectious diseases, does not confer permanent immunity. Also, that just as in other infectious diseases, mild ambulatory forms of the disease are probably far more common than is usually supposed. The outbreaks of yellow fever in West Africa last year, 1910, corroborates this view in a remarkable manner. From May to October there were five outbreaks of yellow fever, viz., at Freetown, Sekondi, Axim, Saw Mills, and Grand Bassam. In the case of four, at least, of these outbreaks no connection could be traced between them, they appeared to originate *de novo*. But more significant still, the first to suffer from the disease and to show the medical authorities that yellow fever was present were the Syrians, small traders who, with *their families*, live in the midst of the natives in the most Stegomyia-haunted parts of the towns. The 1910 outbreaks showed that those who lived outside the native towns remained absolutely yellow fever free. In my opinion, therefore, the evidence is overwhelming that yellow fever is endemic in West Africa, and that the reservoirs are the natives of West Africa. How far the natives of all coast towns in West Africa are reservoirs of the virus, I am not prepared to state, as we require more evidence, but the facts warrant us in stating that in many places, yellow fever is endemic amongst the native inhabitants in a particularly mild form, very much as malaria occurs amongst them. Unfortunately, we have so far no blood or animal test which will prove the presence of the virus, and have only to rely upon a severe case occurring in a non-immune to prove the existence of the disease. In West Africa the non-immune who serves as the test appears to be the Syrian, who happens to live most in contact with the native. From these facts it follows that the great practical lessons to be learnt are that segregation of the non-immunes and Stegomyia destruction are the absolute remedies against yellow fever, also that the answer to the question propounded in the beginning of this paper, viz., why have not the native races in the large towns been decimated or completely wiped out? is that they are completely immunised by mild attacks of yellow fever from childhood. It must also be borne in mind, however, that a considerable proportion of the infantile mortality in the native races may be due to mild yellow fever as well as malaria.

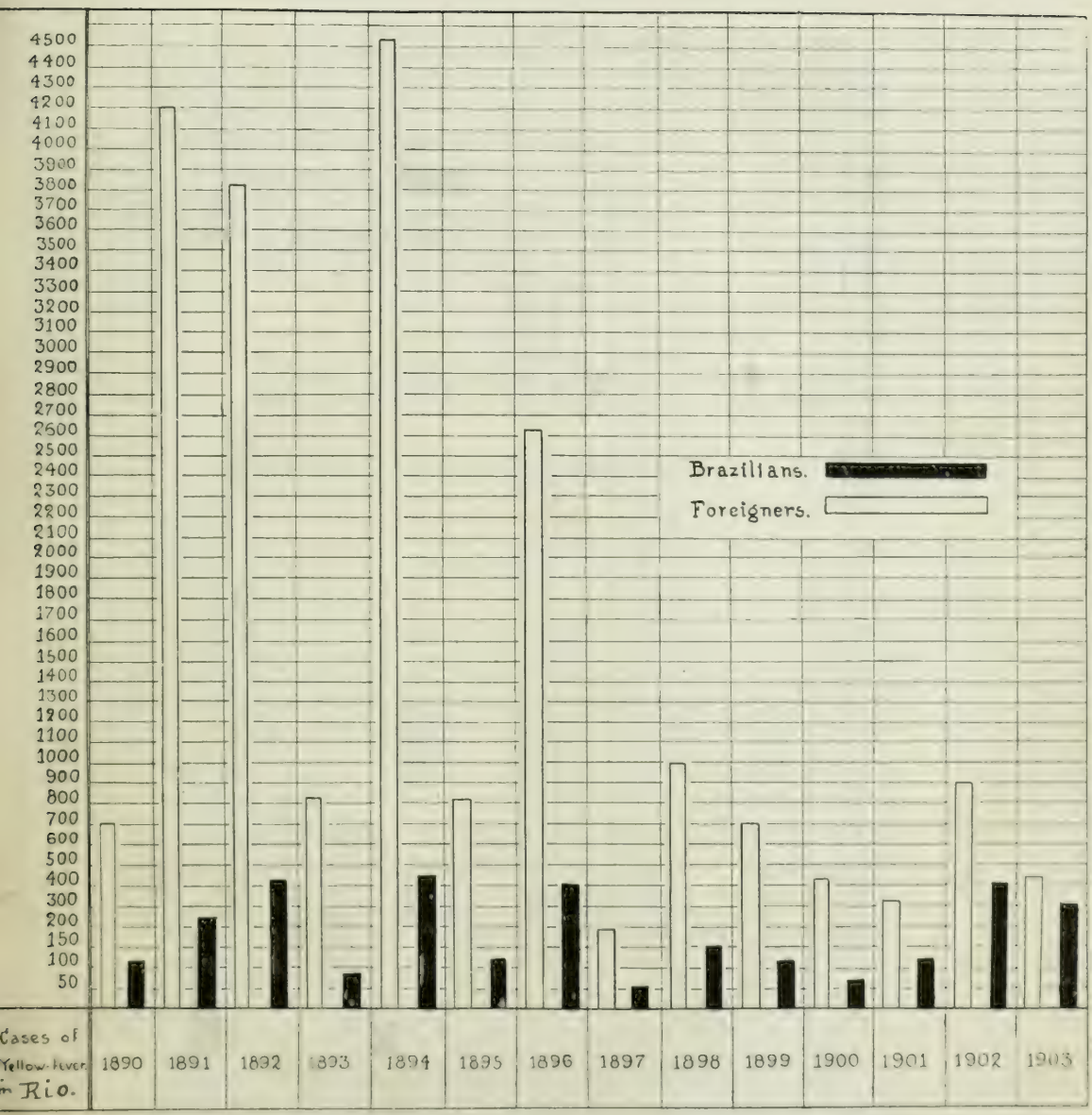


Diagram to illustrate the great difference between the cases of Yellow Fever amongst new arrivals, i.e. foreigners, and the native residents, i.e. the Brazilians in Rio. (Otto and Neumann.)

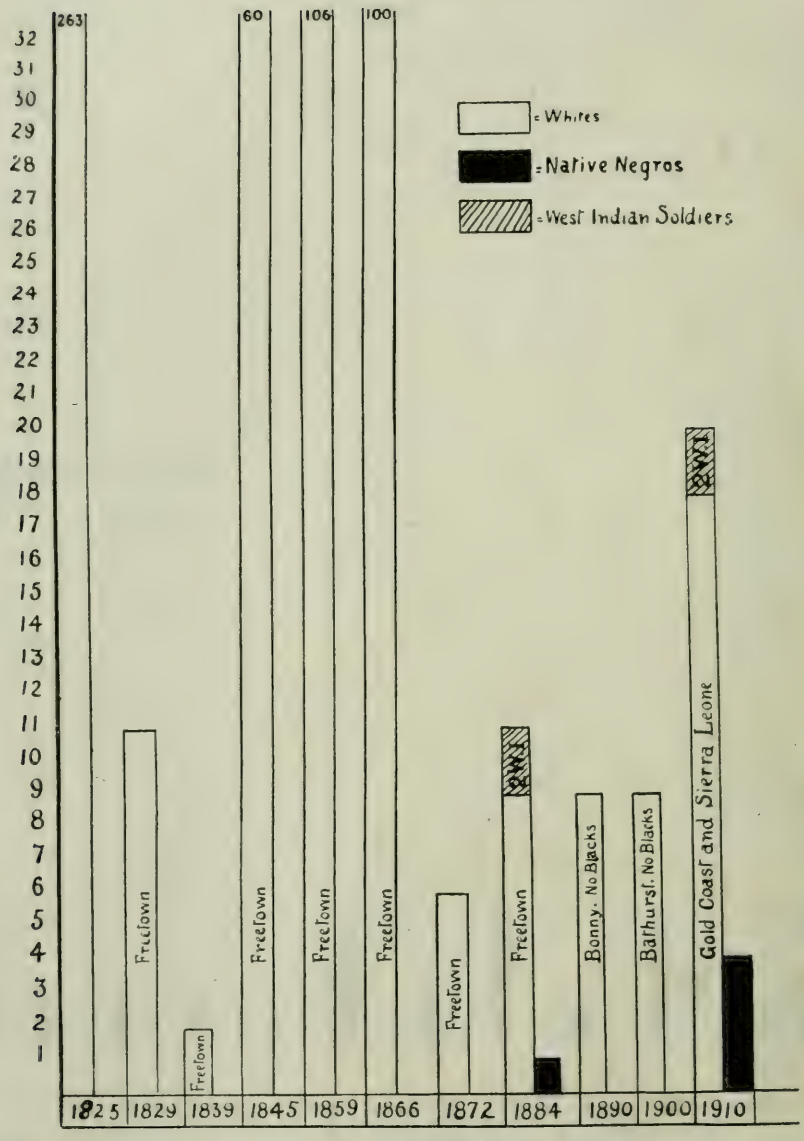


Diagram to show the chief outbreaks of Yellow Fever in British West Africa from 1825–1910. From the year 1884 the cases amongst the natives have been recorded. Note the marked difference between the whites and blacks respectively. Compare it with the Rio table.

ON THE AMOEBAE PARASITIC IN THE HUMAN INTESTINE, WITH REMARKS ON THE LIFE-CYCLE OF *ENTAMOEBA COLI* IN CULTURES

BY

H. B. FANTHAM, D.Sc. Lond., B.A. Cantab., A.R.C.S.

(*Received for publication* 21 *March*, 1911)

INTRODUCTION

The study of the parasitic Amoebae, although of the greatest importance, is one of considerable difficulty. Many investigators, in all parts of the world, have engaged in this study during the last twenty years with the most conflicting results. Consequently, to-day, the utmost confusion prevails as to the pathogenicity, morphology and culturability of the parasitic Amoebae of the human digestive tract, and at present nearly a dozen species are recorded from the human intestine alone. Further, nearly as many more species have been recorded from other organs of man.

Recently, while studying cultures of *Entamoeba coli*, as well as stools from patients being treated for dysentery at the Royal Southern Hospital, Liverpool, I have had occasion to examine the scattered literature on the subject. Before recording the preliminary results of my own studies it will be convenient to set forth a short critical review of the recent work on the parasitic intestinal Amoebae of man, as it seems to me that there has been a tendency to give undue prominence to Schaudinn's researches.

My work has been done in the Liverpool School of Tropical Medicine, under a grant from the Tropical Diseases Research Fund.

THE PARASITIC AMOEBAE OF THE INTESTINE OF MAN

Without adding to the complexity of the subject by a discussion of the history of the association of parasitic amoebae with human dysentery, we may at once give a list, with brief diagnoses, of the species of Amoeba recorded from the human intestine up to the end of 1910. We adopt the generic name *Entamoeba* of Casagrandi and Barbagallo (1895), and divide the parasites into those which are said to be pathogenic and those which are said to be non-pathogenic, beginning with the latter, thus:—

(A) Non-pathogenic forms.

1. *Entamoeba coli* (Lösch, 1875). Diameter, 12 to 25 μ, but variable.

No distinct ectoplasm apparent except at the beginning of pseudopodia formation. Endoplasm granular, filling up the body space when the organism is at rest. Nucleus large, sub-central, spherical, vesicular, containing much chromatin. Nucleus visible in the fresh state. Motility rather feeble.

Multiplication by binary fission or by schizogony, with formation of eight merozoites.

Encystment total and endogenous. Cysts 28 μ in diameter. Sporogony, after nuclear reduction and autogamy, with formation of eight amoebulae.

This parasite lives in the lumen of the large intestine, on the contents thereof; it is incapable of penetrating the mucosa. It may occur in the stools of healthy persons. It is usually considered to be non-pathogenic. Its possible pathogenicity is not above suspicion according to the researches of Billet (1907) and others. It can be cultivated in association with certain bacteria.

2. *E. tropicalis* (Lesage, 1908). This parasite is said to be non-pathogenic, and to occur in the intestine of man in the tropics. Though exhibiting a general resemblance to *E. coli*, and having a nucleus charged with chromatin, it is said to have a clearly distinguishable ectoplasm and to form small cysts (6 to 10 μ in diameter). The small size of the cyst is due to the amoeba having previously divided. Further the nucleus of the cyst is said to break

up into a variable number of daughter nuclei, so that from three to thirteen amoebulae may occur inside a cyst. Several varieties of this species are said to exist by Lesage. It is culturable in symbiosis with bacteria.

3. *E. hominis* (Walker, 1908). Diameter, 6 to 15 μ when at rest. Ectoplasm apparent only in the pseudopodia, endoplasm granular, nucleus circular. A single contractile vacuole present. Encystment total. Cysts small (4·6 to 7·7 μ). Sporulation frequent, spores spheroidal, measuring 0·3 to 0·8 μ.

Culturable with bacteria, but with difficulty. Original strain, now lost, from an autopsy in Boston City Hospital.

This species would appear to be closely allied to *E. tropicalis*.

(B) PATHOGENIC FORMS.

4. *Entamoeba histolytica* (Schaudinn, 1903), also described by Jürgens in 1902.

Diameter, 25 to 30 μ.* Ectoplasm clearly defined. Stout pseudopodia entirely composed of ectoplasm and capable of burrowing into the mucosa and sub-mucosa of the intestine. Nucleus variable in form, excentric and often lateral, poor in chromatin. Nucleus usually invisible in the fresh state. This parasite often ingests red blood corpuscles.

Multiplication by binary fission or by budding. Reproduction by exogenous encystment, giving rise peripherally to minute spores about 3 μ in diameter. The spores become encysted, and, according to Lesage, contain three nuclei.

It is stated that series cultures of this parasite, in association with bacteria, cannot be obtained, at any rate to retain their pathogenicity. Lesage (1907), however, claims to have cultured the parasite in leucocytic exudation from the peritoneum of infected guinea-pigs. The parasite has been found in cases of liver abscess and dysentery in Egypt, China and Japan.

5. *Entamoeba* sp., cultivated by Noc (1909), from cysts derived from liver abscess, from dysenteric stools and from the water supply of Saigon, Cochin China. Noc cultivated this amoeba in association with bacteria. It is, apparently, pathogenic, closely

* Hartmann (1909) gives a smaller size, 15-20 μ.

allied to *E. histolytica*, perhaps showing more marked *internal* budding (schizogony) than *E. histolytica* (judging by Noc's figures). It exhibits polymorphism, and may be a separate species, but is unnamed.

6. *E. tetragena* (Viereck, 1907), synonym *E. africana* (Hartmann).

Recorded from dysenteric cases in various parts of Africa, Brazil and India.

Diameter, 20 to 30 μ, according to Viereck.

Although the trophozoite of this amoeba bears a general resemblance to that of *E. coli*, yet it is said by Hartmann to possess a distinct ectoplasm which is only clearly visible when a pseudopodium is protruded. However, its granular endoplasm may contain ingested red blood corpuscles. There is a large round nucleus visible in the fresh state. Chromidial masses occur in the cytoplasm.

Multiplication proceeds by binary fission.

Sexual reproduction by endogenous encystment, which is preceded by nuclear division into two, reduction and then autogamy. The cysts contain four nuclei.

E. tetragena is pathogenic to man and to kittens, but the dysentery resulting is said to be more benign than that resulting from *E. histolytica*, and liver abscess is said to be rare.

E. tetragena is not culturable.

Hartmann (1909) stated that *E. histolytica* is a rare amoeba, and that in nearly all cases of amoebic dysentery the *E. tetragena* of Viereck is found.

Personally I have met with a case of chronic dysentery under treatment in Liverpool (probably infected in Nigeria), the parasite obtained from the stools being *E. tetragena*.

7. *E. phagocytoides* (Gauducheau, 1908).

This parasite was discovered in a case of dysentery at Hanoi, Indo-China. The amoeba is very small, 2 to 15 μ in diameter. It is active, and possesses a well-developed ectoplasm. It ingests bacteria and red blood corpuscles, while peculiar spirilla-like bodies are found in its cytoplasm.

It multiplies by binary and multiple fission.

Young cultural forms of this amoeba inoculated intravenously into a dog produced dysentery.

8. *E. minuta* (Elmassian, 1909). Found in association with *E. coli*, in a case of chronic dysentery in Paraguay.

It resembles *E. tetragena*, but is smaller, rarely exceeding 14 μ in diameter. No differentiation between ectoplasm and endoplasm. Nucleus invisible in fresh preparations, and when stained is richer in chromatin than that of *E. coli*.

Multiplication by schizogony into four merozoites.

Encystment total and endogenous, giving rise to cysts containing four nuclei, after nuclear reduction and autogamy.

9. *E. nipponica* (Koidzumi, 1909). Found in the motions of Japanese suffering from dysentery or from diarrhoea, in the former case in company with *E. histolytica*. It is also said to occur in healthy persons.

Diameter, 15 to 30 μ.

Clear distinction between ectoplasm and endoplasm. Pseudopodia are not spinose, but are lobopodia. Endoplasm vacuolated, and phagocytic for red blood corpuscles. The nucleus is well defined, and can be seen in the fresh condition; it is rich in chromatin, resembling that of *E. coli* and *E. tetragena*.

Multiplication by binary fission and by schizogony into six or eight merozoites.

Encystment total, and accompanied by formation of chromidia. The complete stages of sporogony have not been followed.

Experiments are necessary to determine the pathogenicity and culturability of this amoeba.

10. *E. undulans* (Castellani, 1905). Found, in company with other Protozoa, in the faeces of persons suffering from diarrhoea in Ceylon.

Diameter, 12 to 30 μ. There is an undulating membrane present, and long straight pseudopodia which appear rapidly, but only one pseudopodium is protruded at a time. Cytoplasm not differentiated into ectoplasm and endoplasm.

Obviously, further knowledge of this parasite is needed.

DIAGNOSIS TABLE

We may tabulate some of the various characteristics said to be diagnostic of the Entamoebae already mentioned thus:—

	Size	Ectoplasm and Endoplasm	Nucleus	Multiplication	Reproduction	Pathogenicity	Culturability
E. coli	12—25μ but variable	No distinct ectoplasm, except at beginning of pseudopodia formation	Round, vesicular, subcentral, with a karyosome. Visible in life	Binary fission. Schizogony—8 merozoites	Encystment total, endogenous, 8 spores in cyst	—	+
E. histolytica ...	25—30μ	Distinct. Endoplasm ingests red blood corpuscles. Burrowing pseudopodia	Small, excentric, poor in chromatin. Invisible in life	Binary fission. Budding	Small exogenous spores	+	—
E. tetragena.....	20—30μ	Ectoplasm distinct when pseudopodia formed. Endoplasm ingests red blood corpuscles	Round. Visible fresh	Binary fission	Encystment total, endogenous. Cysts contain 4 nuclei	+	—
E. tropicalis.....		Distinct	Round, rich in chromatin	Binary and multiple fission (?)	Encystment total, endogenous, numerous small spores in small cyst	—	+
E. hominis	6—15μ	Ectoplasm apparent only in the pseudopodia. Single contractile vacuole present	Round	'By division and by sporulation'	Cysts 4·6—7·7μ	?	+
E. phagocytoides	2—15μ	Ectoplasm well developed. Active. Phagocytic		Binary fission. Schizogony		+	+
E. minuta	14μ	Not distinct	Round, rich in chromatin. Invisible in life	Schizogony—4 merozoites	Encystment total, endogenous. Cysts with 4 nuclei	+	—
E. nipponica ...	15—30μ	Distinct	Round, rich in chromatin. Visible fresh	Binary fission. Schizogony —6 to 8 merozoites	Encystment total. Incompletely known	?	?

NOTE.—*E. histolytica* differs from all the others in that its encystment is exogenous, not total and endogenous as in the other cases where the sporogony is known. From this table it will be seen at once how slight are many of the differences between the so-called species of *Entamoeba* in the human intestine.

SOME CRITICAL REMARKS ON THE VARIOUS INTESTINAL AMOEBAE OF MAN

It is now usually recognised, since the experimental researches of Schaudinn (1903) and others, that amoebae of two kinds may occur in the human digestive tract, namely, pathogenic ones and others which are non-pathogenic. To the latter class belong *Entamoeba coli* and *E. tropicalis*. In the former class must be placed *Entamoeba histolytica* and *E. tetragena*. Further, the non-pathogenic forms are culturable with symbiotic bacteria, while the pathogenic ones are not so culturable, or doubtfully so.

Musgrave and Clegg (1904) were the pioneers of successful modern cultural methods as applied to amoebae. These distinguished workers, however, suggest that 'all amoebas [in the intestine] are, or may become pathogenic,' and state that 'amoebas cultivated from various sources, including the dysenteric intestine, the Manila water-supply, lettuce, etc., have proved pathogenic under certain conditions, which reverses the view held of some of those formerly considered harmless.'

The discrepancy between Musgrave and Clegg's results and those of Schaudinn is usually ascribed to impurity of cultures, due to the presence, unnoticed, of the small cysts of *E. histolytica* in the cultures of the Philippine observers. Lesage (1908) considers that Musgrave and Clegg cultured chiefly *E. tropicalis*, while Werner (1908) thinks that they had *Amoeba limax* in their cultures, and he similarly criticises Walker. But we must still carefully consider Musgrave and Clegg's results, especially when we remember that the possible pathogenicity of *E. coli* is not above suspicion (cf. Billet, 1907), and that quite recently (November, 1909) Elmassian asks how we are to interpret the occurrence of *E. histolytica* in non-dysenteric natives in Asia. Obviously, many further researches are needed.

Regarding the large number of species of parasitic amoebae recorded from the human intestine, I think that few of the so-called species are really good ones. With respect to plurality of species, we must carefully consider the phenomenon of *polymorphism*, a phenomenon markedly exhibited by amoebae. Many of the species are apparently only separated by slight morphological differences,

such as in the distinctness or otherwise of the ectoplasm from the endoplasm, in the structure of the nucleus, or even in size. Such differentiation is unsatisfactory, as must be evident to any investigator who has worked for any length of time on *one* species of organism, who has fixed and stained it by various methods from day to day, and compared the results obtained. Morphological variation, then, must not be overlooked when separating species. Both Musgrave and Clegg (1906) and Noc (1909) have recorded the occurrence of variability in morphological characters of amoebae in cultures started from a single, isolated cyst.

Certain observers, again, have cultivated species of amoebae, and then omitted to test the pathogenicity or otherwise of the cultural forms by experiments on animals. Further, the life-cycles of cultural amoebae must be carefully examined and compared with the natural forms, a point which appears to have been largely overlooked.

It seems to me that the methods of reproduction supply the most valid grounds on which to base species differences, taken in conjunction with possible pathogenicity. We then have three fairly well recognised species of amoebae parasitic in the human intestine, namely:—

(1) *Entamoeba coli*, with its varieties *E. tropicalis* and possibly *E. hominis*. These are apparently non-pathogenic. The encystment is total and endogenous.

(2) *E. histolytica*, the pathogenic agent in certain cases of dysentery and liver abscess recorded from Egypt and China, and perhaps from Europe. The encystment is not total but exogenous, and minute spores are produced. The organism has been best studied by Schaudinn (1903), Craig (1908) and Hartmann (1909).

(3) *E. tetragena*, the pathogenic agent of dysentery in cases recorded from various parts of Africa, Brazil and India. The encystment is total and endogenous. The organism has been studied by Viereck (1907) and Hartmann (1908).

Probably *E. minuta* is merely a variety of *E. tetragena*, while *E. nipponica* seems to belong, as a variety, either to *E. coli* or to

E. tetragena. The position of *E. phagocytoides* is unsatisfactory until its sporogony has been investigated.

Gauducheau (1909) states that at a later stage of the culture the organisms (*E. phagocytoides*) are difficult to keep alive, and then are only about 1 μ in diameter. Brown (1910) considers that Gauducheau's organism clearly shows affinities with *E. histolytica*, like Noc's entamoeba.

Morphologically *E. coli* and *E. tetragena* are somewhat alike and form endogenous cysts, though the daughter forms within the cyst are eight and four respectively. It is interesting to note that Viereck (1907) first thought that *E. tetragena* was a variety of *E. coli*. Our knowledge of *E. tetragena* is not yet quite complete.

PRELIMINARY NOTE ON THE LIFE-HISTORY OF *ENTAMOEBA COLI* AS SEEN IN CULTURES

Two separate cultures of *Entamoeba coli* have been examined. They were derived from dysenteric cases from Manila, and have been maintained on Musgrave and Clegg's medium (at 20° to 25° C.) by sub-inoculations for some three years. I have much pleasure in thanking Dr. Stephens for the material.

The life-cycle of the parasite in cultures has been studied, a point which does not appear to have been fully recorded in previous literature.

The results, not yet complete, may be summarised as follows. On Musgrave and Clegg's medium the amoebae leave their cysts after rupturing them, and some discarded empty cysts may be found in preparations. Sometimes, however, the encysted parasite first appears to swell up or grow, the cyst wall gradually becoming thinner meanwhile until it appears ultimately to be absorbed. Small vacuoles may occur in the cyst. The amoeba when first free usually contains one or two small vacuoles which after combining slightly enlarge and travel to the periphery. This vacuole aids in the protrusion of the first pseudopodium. The pseudopodia are composed chiefly of ectoplasm, though endoplasm flows in to some extent later. The greater part of the body of the amoeba is composed of granular endoplasm, and some of the larger granules may stain metachromatically with methylene blue *intra vitam*. The

amoeba now feeds, grows and moves about in a restricted area which is often approximately circular. The nucleus of the amoeba is round and vesicular with a central karyosome, and is clearly visible in life. There is a clear area in the endoplasm around the nucleus. The parasite at this stage may be called a trophozoite, in preference to the term 'vegetative' stage which is so often used. The amoeba divides by binary fission with nuclear promitosis, in which the karyosome plays an important part. The parasite also divides, occasionally, by schizogony, forming eight merozoites.

On the culture-media which I have been using, the amoebae begin to encyst in about four days, the cyst wall of each being formed by differentiation at the periphery of the now rounded amoeba. The encystment is total. The cyst at first contains a centrally-placed nucleus, with a karyosome. Inside some of the cysts division occurs, and eight daughter forms are produced. The cytological details of sporogony are now being studied, and will be published later. When all the amoebae in a culture have encysted and remained in that condition for some time a new culture must be prepared. The cultures with which I have been working are renewed about every fortnight or three weeks.

I have tried the action on *Entamoeba coli* of some of Dr. H. C. Ross's 'auxetics'—substances capable of inducing division in living cells. I wish to thank Drs. H. C. Ross and J. W. Cropper for providing me with some of these substances. Many of these auxetics occur naturally in the body, and my attention has been especially directed to some which are found in the intestines, such as tyrosin, leucin and skatol.

These substances are best used in a jelly with agar, sodium chloride and alkali (sodium bicarbonate), forming a slightly alkaline culture medium. When such a medium, containing about 0·2 per cent. of tyrosin, is inoculated with cysts of *E. coli* obtained from a culture on Musgrave and Clegg's medium, the period of the life-cycle is shortened, and the amoebae on the culture reproduce for several generations. I have a culture which has already gone through five generations. Somewhat similar results occur on a culture-medium containing a similar quantity of leucin. Unfortunately the 'growth' does not increase much, for there is

apparently rapid death of some of the amoebae, probably resulting from insufficient food-supply in the media.

One interesting and novel result on tyrosin-containing media, compared with cultures on Musgrave and Clegg's medium, is that a complete life-cycle of *E. coli* is passed through in about three days (at 20° to 25° C.), when all the amoebae of a given generation have encysted. Then a large number of the cysts produce eight daughter forms inside them, and the amoebulae come out of the cysts and start a new generation on the same medium. I have seen these phenomena continued through five generations, whereas on Musgrave and Clegg's medium only one generation of amoebae is usually produced, and few of the cysts give rise to eight daughter forms. Further, binary fission of amoebae occurs on a tyrosin-containing medium more frequently than on a Musgrave and Clegg medium. The process of binary fission involves a primitive mitosis (or promitosis) of the nucleus, caps of chromatin derived from the karyosome being formed at the ends of the rudimentary spindle. Stages of schizogony have also been seen.

Skatol added to a preparation containing free *E. coli* rapidly induces encystment. This is of interest since skatol occurs naturally in the hinder part of the digestive tract and in the faeces.

Auxetics such as supra-renal extract and metaphenylenediamine also apparently induce division. The binary fission in such cases is of the nature of unequal promitosis, probably due to the rapidity with which it is induced.

These researches are being continued, and I hope to publish a more detailed and illustrated account later, in which the cultural forms will be compared with the amoebae in their natural habitat.

REFERENCES

The following list is confined principally to papers published during the last ten years. Only a few of the older papers are included, but further references to these may be obtained at the end of some of the memoirs listed.

Bensen, W. (1908), ' Die Darmprotozoen des Menschen.' Arch. f. Schiffs- u. Trop.-Hygiene, Bd. XII, pp. 661-676.

Billet, A. (1907), ' Sur un cas de dysenterie " nostras " à Amibes.' C. R. Soc. Biol., LXII, p. 1232.

Brown, W. C. (1910), ' Amoebic or Tropical Dysentery, its Complications and Treatment.' 271 pp. London, Bale & Danielsson.

Casagrandi, O. e Barbagallo, P. (1897), ' *Entamoeba hominis* s. *Amoeba coli* (Lösch).' Ann. d'Igiene speriment., VII, pp. 103-166, 1 pl.

Castellani, A. (1905), ' Observations on some Protozoa found in Human Faeces.' Centralbl. f. Bakteriol. u Parasitenk., Abt. 1, Orig., XXXVIII, pp. 66-69.

Councilman, W. T. and Lafleur, H. A. (1891), ' Amoebic Dysentery.' Johns Hopkins Hosp. Reports, II, pp. 395-548, 7 pls.

Craig, C. F. (1908), ' Studies upon the Amebae in the Intestine of Man.' Journ. Infect. Diseases, V, pp.324-377, 2 plates.

Elmassian, M. (1909), ' Sur une nouvelle espèce amibienne chez l' homme, *Entamoeba minuta*, n. sp.' Centralbl. f. Bakteriol., Abt. 1, Orig., LII, pp. 335-351, 2 plates.

Gauducheau, A. (1908), ' Culture d'une amibe dysenterique ' (*E. phagocytoides*), C. R. Soc. Biol., LXIV, p. 493.

Gauducheau, A. (1909), ' Sur une culture amibienne,' Bull. Soc. Path. Exotique, II, pp. 247, 370, 568.

Hartmann, M. (1908), ' Eine neue Dysenterieamöbe, *Entamoeba tetragena* (Viereck), syn. *Entamoeba africana* (Hartmann),' Arch. f. Schiffs- u. Trop.-Hygiene, Bd. XII, Beiheft 5, pp. 117-127.

Hartmann, M. (1909), ' Untersuchungen über parasitischen Amöben. I. *Entamoeba histolytica* Schaudinn.' Arch. f. Protistenkunde, XVIII, pp. 207-220, 1 plate.

Jürgens (1902), ' Zur Kenntnis der Darmamöben der Amöben-enteritis.' Veroff. a.d. Gebiete d. Militär-Sanitätswesens, XX, p. 110.

Koidzumi, M. (1909), ' On a new parasitic Amoeba, *Entamoeba nipponica,* found in the intestine of Japanese,' Centralbl. f. Bakteriol., Abt. 1, Orig., LI, pp. 650-653.

Lesage, A. (1905), ' Culture de l'Amibe de la Dysenterie des pays chauds,' Ann. Inst. Pasteur, XIX, pp. 9-16, 2 plates.

Lesage, A. (1907), ' Culture du parasite de l'amibiase humaine (Dysenterie amibienne),' C. R. Soc. Biol., LXII, pp. 1157-1159.

Lesage, A. (1907), ' L'amibiase chez le chat (Dysenterie amibienne),' C. R. Soc. Biol., LXII, pp. 1191-1193.

Lesage, A. (1908), ' Note sur les entamibes dans la dysenterie amibienne des pays chauds,' Bull. Soc. Path. Exotique, I, pp. 104-111.

Lösch, F. (1875), ' Massenhafte Entwickelung von Amoeben ein Dickdarm,' Virchow's Archives, LXV, pp. 196-211.

Musgrave, W. E. and Clegg, M. T. (1904), ' Amebas : their Cultivation and Etiologic Significance,' Dept. of Interior, Bureau of Govt. Labs., Biol. Lab., Manila, No. 18.

Musgrave, W. E., and Clegg, M. T. (1906), ' The Cultivation and Pathogenesis of Amoebae,' Philippine Journ. of Sc., I, pp. 909-950.

Noc, F. (1909), ' Sur la Dysenterie amibienne en Cochinchine,' Ann. Inst. Pasteur, XXIII, pp. 177-204, 4 plates.

Schaudinn, F. (1903), ' Untersuchungen über die Fortpflanzung einiger Rhizopoden,' Arb. a. d. Kaiserl. Gesundheitsamte, XIX, Heft 3, pp. 547-576 (see p. 563).

Viereck, H. (1907), ' Studien über die in den Tropen erworbene Dysenterie,' Arch. f. Schiffs- u. Trop.-Hygiene, Bd. XI, Beiheft 1, pp. 1-41, 3 plates.

Vincent, H. (1909), ' Note sur la latence prolongée de l'Amibe dysenterique dans l'intestin humain. Les " porteurs d'amibes." ' Bull. Soc. Path. Exotique, II, pp. 78-80.

Walker, E. L. (1908), ' The parasitic Amebae of the Intestinal Tract of Man and other animals,' Journ. Med. Research, XVII, pp. 379-459, 4 plates.

Werner, H. (1908), ' Studien über pathogene Amöben,' Arch. f. Schiffs- u. Trop.-Hygiene, Bd. XII, Beiheft 11, 18 pp., 6 plates.

SOME FURTHER OBSERVATIONS ON THE TSETSE-FLY, DESCRIBED IN THESE ANNALS AS *GLOSSINA GROSSA*, Etc.

BY

R. NEWSTEAD, M.Sc., A.L.S., &c.

(*Received for publication* 29 *March*, 1911)

I have recently ascertained that Bigot's type of *Glossina grossa* is preserved in the British Museum (Natural History) at South Kensington, and that it is morphologically distinct from the tsetse-fly which I described under this name in December, 1910.

Had I known at the time that the type was available, I should have taken steps to have verified my conclusions, and thereby have avoided the confusion in the synonomy of this insect. Now that I have had an opportunity of examining Bigot's type, I have no hesitation in stating that my examples are specifically distinct, and the name *nigrofusca* which I suggested must now be adopted, the synonomy of which is appended below:—

Glossina nigrofusca, Newstead. Annals of Tropical Medicine and Parasitology, Vol. IV, 3, pp. 370, 373, 1910.

Glossina grossa, Newstead (*nec* Bigot). Annals of Tropical Medicine and Parasitology, Vol. IV, 3, p. 373, 1910.

The distinguishing characters of this insect are that the terminal segment of the antenna is much more strongly recurved at the tip than in *G. grossa*; it is also clothed with much longer hairs; the thoracic markings are more sharply and clearly defined, and the general colour of the insect is darker than in any other species of the *fusca* group.

Glossina palpalis, var. *wellmani*, Austen.

Mr. E. E. Austen has very kindly afforded me the opportunity of examining a para-type male of this variety of *G. palpalis*, and I find that the morphological characters of the armature are specifically the same as in *G. palpalis*. The example in question bears the following data:—'Katumbela River, Benguella, Angola, November, 1904, Dr. F. C. Wellman. 1906. 139.'

ON THE CORRELATION BETWEEN TRYPANOSOMES, LEUCOCYTES, COAGULATION TIME, HAEMOGLOBIN AND SPECIFIC GRAVITY OF BLOOD

BY

VISHNU T. KORKE, M.R.C.P., D.T.M.,

JOHNSTON COLONIAL FELLOW, THE UNIVERSITY OF LIVERPOOL

(*Received for publication* 4 *April*, 1911)

During daily observations of the blood of animals, viz., guinea-pigs and rats infected with *T. gambiense* and *T. rhodesiense*, it was observed that the coagulation rate of blood and the rate of haemolysis by a dehaemoglobinising fluid on thick films varied from time to time.

Wright's method was used in determining the coagulation time of blood at the temperature of half blood heat. In taking blood, squeezing of the tissues was carefully avoided. Leucocytes and parasites were counted by Ross and Thomson's* quarter millimetre pipette method on thick films, using an Ehrlich's eye-piece. They were counted on the same film and in the same field in specimens stained by Romanowsky's method. Many parasite counts were made by Dr. H. B. Fantham, to whom my thanks are due. The haemoglobin percentage value was determined by Sahli's haemoglobinometer.

The specific gravity was determined by Hammerschlag's benzol-chloroform method, a modification of Roy's. In the case of infected animals, the specific gravity was always below 1,060. In the case of control animals I found the specific gravity was rarely below 1,055, and as the instrument was not graduated above 1,060, readings above this were expressed as 'little above,' 'well above,' or 'far above' the 1,060 mark. The observations were made every twenty-four hours, practically at the same hour of the day, three hours after

* Annals of Tropical Medicine and Parasitology, Vol. IV, p. 268 (1910).

feeding the animals, viz., guinea-pigs and rats infected with *T. gambiense* and *T. rhodesiense*.

I am indebted to Walter Stott, Esq., Honorary Statistician to the Liverpool School of Tropical Medicine, for calculating the following correlations. This. and the application of correlation method to other observations, is one of the first attempts at applying precise mathematical method to clinical investigations. From 173 observations on specific gravity, haemoglobin and parasites, the following results were obtained:—

(1) Correlation between the number of parasites and the amount of haemoglobin—

$$r = 0{\cdot}1517 \pm 1902, \text{ error greater than } r.$$

Result = No correlation shewn.

(2) Correlation between the number of parasites and specific gravity of blood—

$$r = 0{\cdot}2530 \pm 1899, \text{ error only slightly less than } r.$$

Result = No correlation shewn.

(3) Correlation between the amount of haemoglobin and specific gravity—

$$r = 0{\cdot}9956 \pm 0{\cdot}0049.$$

Result = Strong correlation.

TABLE showing the mean specific gravity value and amount of haemoglobin in different animals infected with trypanosomes.

Animals			Mean specific gravity of blood	Mean haemoglobin value	No. of observations	Remarks
Guinea-pig	1.	*T. gambiense* ...	1039·8	65·9 %	26	
,,	2.	,, ...	1048·7	82·9 %	32	
,,	3.	,, ...	1043·3	76·1 %	39	
,,	1.	*T. rhodesiense*...	1050	83·8 %	19	
,,	2.	,, ...	1046·25	85·6 %	8	
,,	3.	,, ...	1047·65	79·7 %	19	
,,	4.	,, ...	1051·2	96·2 %	4	
Rat	1.	*T. rhodesiense*...	1050	87·8 %	7	
,,	2.	,, ...	1047·65	77·5 %	2	
,,	3.	,, ...	1048·7	81·2 %	4	
,,	4.	,, ...	1044·1	72·9 %	12	
,,	5.	,, ...	1060	110 %	1	On the day of inoculation
Total 12				Total	173	

I. PARASITES AND LEUCOCYTES

From 224 observations on twenty-seven animals no correlation was found between the number of parasites and leucocytes per mm.^{3} in animals infected with *T. gambiense* and *T. rhodesiense*. The mean value of leucocytes per mm.^{3} in infected guinea-pigs and rats was found to be 11,700 and 33,800 respectively.

From twenty-four observations on two control animals the mean leucocyte value per mm.^{3} was found to be 9,344 in a normal guinea-pig weighing 800 grammes and 18,863 in a rat weighing 215 grammes.

The normal value of leucocytes per mm.^{3} in guinea-pigs and rats varies according to the age and weight. Leucocytes appear to be abundant—(*a*) during the incubation period; (*b*) when parasites have temporarily disappeared; (*c*) towards the end of infection. Leucocytic values in infected animals may vary for the following reasons:—(i) Owing to osmotic disturbances between the tissue fluids and lymph and blood, dilution and concentration of the blood plasma giving rise respectively to *apparent* leucopenia or leucocytosis. *(ii) It is probable where the infection is lymphatic that this may give rise to what may be called 'passive lymphocytosis'; for when the leucocytes appeared to be abundant, the majority of them were lymphocytes. But whether this was an 'active' or 'passive' lymphocytosis remains to be seen.

II. PARASITES AND THE COAGULATION TIME OF BLOOD

From 118 observations on twenty-three infected animals no correlation was found between the number of parasites and the coagulation time of blood. The mean value of coagulation time in infected guinea-pigs and rats was found to be three minutes eleven seconds and five minutes one second respectively.

From twenty-four observations on two control animals the mean coagulation time was found to be four minutes fourteen seconds in guinea-pigs, and three minutes thirty-six seconds in rats.

III. PARASITES AND HAEMOGLOBIN

From 291 observations in thirty-five infected animals no correlation was found between the number of parasites and the

* Lazarus Barlow. Experimental or General Pathology, 1904, p. 154.

percentage of haemoglobin. The mean value of Hgb. in infected guinea-pigs and rats was found to be 81 per cent. and 80 per cent. respectively.

From fifty-one observations in eleven control animals it was found that the haemoglobin percentage was rarely below 95 per cent. In the majority of observations it was between 100 per cent. and 120 per cent.

In animals infected with *T. gambiense* and *T. rhodesiense* there is a fall in the haemoglobin percentage, but it is not of a marked degree, and further it is irregular.

IV. PARASITES AND THE SPECIFIC GRAVITY OF BLOOD

The correlation figures of 173 observations on twelve infected animals have been given earlier.

From forty-two observations on sixteen control animals it was found that there is a fall in the specific gravity of the blood in animals infected with *T. gambiense* and *T. rhodesiense*.

In the majority of the control observations it was found that the specific gravity value was always 'a little above' or 'far above' 1060 mark. It was rarely as low as 1050.

It would be natural to ascribe the cause of the fall in specific gravity to the number of trypanosomes in blood. However, this does not appear to be the case. There is no correlation whatsoever between the number of parasites and the specific gravity values. Consequently one has to look for a cause which is intimately related to the trypanosomes. Such a cause may be the toxins* of trypanosomes. The liberation of these products is under the influence of diverse circumstances, unknown at present. Hence there is no direct clinical method of demonstrating a possible correlation between toxins and specific gravity.

If the liberation of toxin is irregular it would account for the fall in specific gravity, which is irregular also.

As the density of the blood depends on the intra-corpuscular haemoglobin and the blood plasma, and as there is no evidence at

* Used in the sense of product or products of metabolism, disintegration of trypanosomes or the liberation of their toxin.

present of haemolysis *in vivo* in trypanosomiasis, consequently the fall is probably due to dilution of the blood plasma.*

There exists, however, a strong relation between the specific gravity value and haemoglobin percentage under normal conditions.† So strong is this ratio that the specific gravity value can be estimated approximately by determining the haemoglobin percentage. Consequently one might infer the fall in specific gravity is due to the fall of haemoglobin. No doubt a strong correlation exists between them in experimental trypanosomiasis. But from Hammerschlag's table† on specific gravity and Hgb. percentage, one sees that there is a greater fall in specific gravity value than could be accounted for by the haemoglobin percentage. Unless, then, dilution of blood plasma occurs, an explanation is very difficult.

Finally we may note that oedema is a characteristic of human trypanosomiasis. This oedema is not due to those causes which produce oedema in cardiac, renal, pernicious anaemia and cachectic conditions, such lesions are not pathognomonic of human trypanosome infections. The oedema in the latter is fairly early in its onset. In experimental observations it was noticed that the specific gravity value fell from 1,040 to 1,030 when the animals were in the incubation period and when they were showing few trypanosomes (1 to 90 per mm.^{3}).

We may conclude then:—

(1) That the number of trypanosomes in the peripheral blood is not responsible for the fall in specific gravity value.

(2) That the probable cause of the fall is a product, toxic in nature.

(3) That this toxic product damages the osmotic membranes and increases their permeability to the tissue fluids, whereby the blood plasma gets diluted.

(4) That the dilute nature of the toxic blood plasma facilitates the onset of oedema in human trypanosomiasis.

* For a full discussion of this subject, see Lazarus Barlow, Experimental Pathology, 1904, pp. 189-225 and 692-709.

† Cabot, Clinical Examination of Blood, 1897, p. 31.



Elliot & Fry, phot.

Robert Boyce

Volume V August, 1911 No. 2

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY

THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Editor

PROFESSOR SIR RONALD ROSS, MAJOR I.M.S. (RET.), D.P.H., F.R.C.S., D.Sc., LL.D., F.R.S., K.C.B.

In Collaboration with

J. W. W. STEPHENS, M.D., CANTAB., D.P.H.
R. NEWSTEAD, M.Sc., A.L.S., F.E.S., Hon. F.R.H.S.
J. L. TODD, B.A., M.D., C.M. McGill, D.Sc., Liv.
H. WOLFERSTAN THOMAS, M.D., C.M. McGill.
ANTON BREINL, M.U.DR.

C. Tinling & Co., Ltd.
Printers to the University Press of Liverpool
53 Victoria Street

IN MEMORIAM

Professor Sir RUBERT BOYCE, F.R.S.

We deeply regret to record the death of Sir Rubert Boyce, Dean of the Liverpool School of Tropical Medicine, and one of the editors of these Annals. His death, from cerebral haemorrhage, occurred on Friday, June 16th, 1911, at the early age of forty-eight. In 1906 he suffered from a severe attack of hemiplegia, from which it was hardly expected he would recover. Not only did he regain comparative health, but continued to work with the same ardent energy so characteristic of him, and even after a minor attack some weeks before the last fatal one, his indomitable spirit would not be checked. His manifold activities in Liverpool, especially in helping to found the University, are well known to all in this city, and his spirit of progress found wide scope for its play when Mr. Chamberlain, at the Colonial Office, first expounded in practical form the idea of tropical schools for the instruction of medical officers in the Tropics, and for the study of tropical diseases. Sir Rubert at once threw himself with unbounded confidence into the new movement, and, together with Sir Alfred Jones, established the Liverpool School of Tropical Medicine in 1898. It does not become us here to point out what part this School has played in the history of tropical medicine. Sir Rubert was not content with merely starting the movement in Liverpool, but with unremitting interest and zeal devoted the remainder of his short life to tropical medicine. His foresight was remarkable; and to quote only one instance, it was to his eager persistence and untiring effort that we owe the two professorial chairs of Tropical Medicine and Entomology associated with this School. Latterly, the subject of yellow fever more particularly claimed his attention, and in 1905, at the invitation of the American Government, he visited New Orleans to study the epidemic there, and he also visited British Honduras. In 1907 he again set out for the West Indies for the same purpose, and finally, in 1910, to West Africa. The results

of his observations have been published in several official reports; but in order that the public might be interested and instructed in sanitation, he published three works—'Mosquito or Man,' 1909; 'Health Progress and Administration in the West Indies,' 1910; 'Yellow Fever and its Prevention,' 1911—in popular form, which had an immediate and great success.

We need not discuss here his views on yellow fever in West Africa. Whether they be finally accepted or no, certain it is that he focussed attention on the subject in a way which had never been done before, and even before his death practical action was being taken by the Home Government based on his recommendations. There are many in the Tropics who will mourn the loss to the Empire; there are many who will miss his most cheery optimism; there are many who will never know what they have owed to him. For ourselves we must express our deep grief at the loss of a chief of amazing energy, of magnetic inspiration, and beyond everything, one who was a devoted friend. *Vale.*

NOTE ON TROPICAL DISEASES IN SOUTHERN ITALY

BY

PROFESSOR UMBERTO GABBI, OF ROME.

(*Received for publication* 26 *March*, 1911)

For several years clinicians of Sicilian Universities (Gabbi, Giuffrè, Jemma) have called the attention of practising physicians and of the Government to certain diseases, as yet unknown, or very little known, which belong to the great chapter of tropical pathology. The first, which Gabbi, Giuffrè and their pupils made known, was *Mediterranean fever*, which, designated in Palermo by Federici as '*febbre miliare*,' and by Tomaselli in Catania, as '*febbre continua sudorale epidemica*,' was bacteriologically determined by my researches and by those of my pupils, and confirmed, principally, by the subsequent ones of Trambusti, Pollaci and Pulvirenti.

In Naples it had, from 1872, in which year it was discovered, twelve different designations; and only after my communication to the Medical Congress in Rome, in 1906, where, in consequence of a controversy between myself and Castellino, of Naples, aroused by my decisive affirmation that the 'febricola,' or 'Naples fever,' was nothing else than Mediterranean fever, Arnaldo Cantani, junr., undertook bacteriological researches, by which it was proved by Wright's sero-diagnostic method, and by blood culture, that the germ producing the infection was *Micrococcus melitensis*.

In 1901 Zando and Tiberti, in Florence, had isolated a micro-organism, which they identified as that of Bruce; in 1904 it was isolated by Professor Carbone in Pisa, from a patient coming from Catania, and who died in the medical clinic of Professor Queirolo; in 1905, by Cippitelli in Rome. After 1906 the question was much more seriously studied, and, from 1906 to 1910 it was found that Mediterranean fever was widely diffused in Sicily and Southern Italy, and is to be found also in Central Italy, especially in the provinces of Leghorn, Lucca and Florence; and in Upper Italy

(Bologna, Padua, Milan). During the same period (1906-1909) I have, with my pupils, made examinations of goats and have succeeded in demonstrating that amongst us, in Sicily and in Southern Italy, goats are infected, and not only those imported from Malta, but both those of our own country, and also the crossed ones. An approximate estimate, which my pupils are now making, by means of the milk test, in Sicily and Southern Italy, proves the high average of the infection (from 3 to 17 per cent.) in the goats which furnish milk to these populations, as they make use, almost exclusively, of goats' milk.

The symptoms which Bruce's septicaemia present among us are not so grave as those in Malta, and neither so extraordinary as those which our French colleagues describe in cases which, for two years, they have discovered in various departments of Southern France, and also in Paris.

Another disease, believed to be typically tropical, viz., *Kala-azar* was demonstrated five years ago among us by Pianese of Naples. He announced in 1905 at the Congress of Pathology in Rome, that he had found parasites in the spleen of children suffering from *Anaemia splenica pseudoleucaemica*, discovered by Professor Cardarelli in 1880, and afterwards designated by Fede under the name of *Anaemia splenica infettiva*. Two varieties were distinguished, one with fever and one without. Pianese found *Leishmania*, sp., in the first, and, in a paper published more than three years and a half afterwards, agreed with Nicolle's observations, considering it to be a species of *Leishmania*, and on morphological grounds named it *Leishmania infantum*, proposing to change also the name of the disease to *Anaemia infantum a Leishmania*, Pianese. In 1907, during a course of clinical lectures to practising physicians (of Sicily and Calabria), I stated the existence of *Kala-azar* in Sicily; and, in 1908, four months after Pianese's publication, I communicated twelve cases of *Kala-azar*, observed in Messina, Calabria and the Lipari Islands (two with *Leishmania* in the spleen), and afterwards my publications led to a full series of studies, which demonstrated how, in reality, the febrile variety of *Anaemia splenica infantum* is nothing else than *Kala-azar*. Contrary to Pianese's statements I and my scholars demonstrated:—

1. That even youths and adults can be affected by *Kala-azar*, though very seldom.
2. That the disease appears at the beginning of Spring.
3. That it is greatly diffused in littoral towns of the South.
4. That it affected principally the lower classes.

Studies upon the agent of transmission of *Leishmania* are being actively pursued, and, besides the *Cimex lectularius* (Patton, Rogers), and *Conorrhinus rubrofasciatus* (Donovan), Basile implicates also *Pulex serraticeps*. At the same time that I discovered *Kala-azar* in Sicily and Calabria, Basile found *Leishmania* sp. in the dog.

A year afterwards, together with Dr. Lacava, we found cases of Oriental sore in Bovalino (Calabria) and Bordonaro (Messina), and others have observed them in various countries of Reggio Calabria (Palozzi, Bova), and in Catania and Palermo. I had the luck to observe the first example of multiple Oriental sore in a woman of a district near Messina (Tremestieri).

Besides these, typically tropical, sub-tropical, and endemic diseases, I found in Messina, in 1907, and described an epidemic of *Dengue fever* (150 cases) imported by merchants of Tripoli (Africa), and afterwards little epidemics were observed in Francavilla (Messina) and in Bovalino (Calabria).

In 1910 I have clinically recognised the *three days' fever* or *Pappataci fever* in an epidemic which attacked more than 4,000 persons, and which diffused itself along the Ionic Coast of Calabria on its eastern side. Examples of a summer fever, clinically corresponding to the fever caused by *Phlebotomus* had been for some years described by Italian military doctors, but the identity with the *three days' fever* was not yet declared by them before my publications. I think this will prove to be a summer disease which will regularly appear in Upper and Lower Italy; and, if the physicians of Central Italy would pay attention to it they would certainly discover it also.

I have observed and described undoubted cases of climatic bubo; Dr. Lacava has recently found in Calabria cases of *Ulcum tropicum* and of *Myiasis ocularis*.

The above-mentioned diseases are to be found likewise in North Africa, and this community of diseases is explicable if we consider

that Arabs ruled for years in Sicily and Calabria; that commercial intercourse was, and is, ever more active between Sicily and Calabria and the Italian colonies of North Africa; that, moreover, intermarriage has taken place between Italians and women of African colonies. These data explain this area of common pathology, and so much the more when we consider that the climate and vegetation of North Africa and Sicily differ so very little, permitting of the existence of similar parasites, and, again, the habits of the classes which are the most affected by the described diseases are very alike.

These studies, begun in the Medical Clinic of the University of Messina, are now being continued, since the earthquake, in the Medical Clinics of Professor Baccelli in Rome, with the aid of the Government.

LITERATURE

'Studi intorno alle malattie tropicali della Calabria e Sicilia.' Fasc. I, II, III.
'Malaria e malattie dei paesi caldi.' Edited by Prof. U. Gabbi. Rome, 1911.

THE PAPATACI FLIES (*PHLEBOTOMUS*) OF THE MALTESE ISLANDS*

BY

R. NEWSTEAD, M.Sc., A.L.S., &c.

(PLATES V—VII.)

(*A report of the twenty-third Expedition of the Liverpool School of Tropical Medicine.*)

(*Received for publication* 15 *May*, 1911)

Acting under the instructions of the Liverpool School of Tropical Medicine I proceeded to Malta on the 25th of June, 1910, and stayed in the Island for a period of two months. The object of this expedition was to investigate the problems connected with the menace to health caused by the blood-sucking 'Papataci Flies' of the genus *Phlebotomus*. † The greater part of my time was devoted to searching for the breeding-places of these insects with a view to devising practical prophylactic measures for the control of the pest. Other phases relating chiefly to the bionomics of *Phlebotomus* were also investigated; and attempts were made to rear the insect from the egg.

On making a critical examination of the material collected during the first week of my visit, two distinct species (*P. papatasii*, Scop., and *P. perniciosus*, n. sp.) were found to be almost equally abundant; and examples of a third, though apparently rare, species (*P. minutus*, Rond.) were subsequently taken. Since my return to England, Captain P. J. Marett, R.A.M.C., has very generously placed the whole of his collection of Maltese Papataci flies in my hands for examination and report; and among the numerous examples there were two specimens which have proved to be a new and hitherto undescribed species (*P. nigerrimus*, n. sp.), so

* Reprinted from the Bulletin of Entomological Research, Vol. II, pt. 1, pp. 47-78, 1911.

† These insects are generally known to Englishmen as 'Sand Flies.'

that altogether four distinct species of *Phlebotomus* are now known to occur in the Maltese Islands.

These discoveries, though of much interest for the zoologist, add considerably to the labours of those who are or may be engaged in studying these insects more especially from a medical point of view; as owing to the minute morphological differences which exist between the females of these small midges the task of separating the respective species, more especially the commoner ones, is one which can be accomplished only after long and careful microscopical examination and comparison.

Hitherto the only species recorded from Malta was the common and widely distributed *P. papatasii*; but judging from recent experience, I have come to the conclusion that the almost equally abundant *P. perniciosus* must have been seen, though not recognised, by those who have been engaged in studying the bionomics of these insects.

It is highly probable too, that examples of this species were also used by those who conducted the transmission experiments, and although one has no direct proof, it is possible that *P. perniciosus*, like its near relative (*P. papatasii*), may also act as a carrier of Papataci fever.

THE SEARCH FOR BREEDING PLACES OF PHLEBOTOMUS

The results of my unremitting search for the breeding places of these insects were that I secured two larvae from the crevices of the loose rock in the 'caves' or catacombs at Notabile near the centre of Malta; thereby confirming the discoveries made by Captain Marett[6]* a month or so previously. Had my searches been continued in the same kind of habitat I have reason to believe that a few more larvae would have been secured, but having trained the eye so as to facilitate the finding of so minute an object the more readily on any future occasion, I proceeded in other directions, and searched innumerable places that were thought likely to form suitable breeding grounds for these insects, unfortunately without discovering either eggs, larvae or pupae; disappointment met me at

* Such numbers refer to the bibliography on page 181.

every turn and I am therefore unable to add anything that is new or noteworthy regarding the breeding places of *Phlebotomus papatasii* or any of the allied species.

In addition to the cave from which larvae were secured I also inspected the places in which both larvae and pupae had been found by Captain Marett; these were the cave at Gozo, the embankment forming part of the Cottonera Lines, and the stone wall in Captain Marett's garden, which he had thoroughly explored and had also kept under close and constant observation for a considerable time. In all of these places the conditions were very similar, if not almost identical.

In the caves the larvae occurred in the crevices and fissures beneath the loose rock amongst the damp earth, etc., at some distance from the surface, and I was informed that those which were found in the stone wall, occurred low down near the foundations, well within the centre, and attached chiefly to the under surface of the stones; while those from the Cottonera embankment were found at some considerable distance from the surface, where the stones were damp[6].

The crevices between the loose rock in the caves were often found partly filled with soil rich in organic remains. In the caves at Notabile, in which the larvae were found, the soil had for the most part been reconstituted by the burrowing larvae of various insects and other allied animals. To such an extent had this been done in some instances that quite 50 per cent. of the deposit consisted of the rejectamenta of insects, woodlice (*Oniscus* sp.), etc. Here and there were found also large numbers of the empty pupae of *Stomoxys calcitrans* and the pupae of other Muscid flies whose larvae had matured in the stable refuse which had been stored in the cave for agricultural purposes.

In all of these places the conditions were practically the same, the three main factors being: (*a*) the presence of organic matter; (*b*) moisture, but not in excess; and (*c*) the absence of light.

The principal places which were searched as being likely to afford suitable breeding-grounds for Papataci flies were as follows: The main sewers and ventilating shafts in various parts of the city of Valetta; drains of various kinds, cesspools and latrines in many places; cellars and prison cells in the Police Court; sewage works,

and the dark damp buildings used by the Customs as bonded stores; refuse of all kinds, especially such as occurred in dark damp places; the refuse 'tips,' and the roots of plants along the coast, especially in localities which were known to be badly infested with the flies; the decayed stems of the Prickly Pear (*Opuntia* sp.); collections of stone and rock in shady places in gardens and elsewhere; freshly excavated earth and rock; the empty shells of molluscs (chiefly *Helix* sp.) found in caves and other sheltered situations; refuse in caves which were used as stables for oxen and other domesticated animals, and the faecal matter which was found in those which had been used as latrines; the roots of trees, ivy and flowering plants which were kept moistened by constant supplies of water, also those growing in the rock fissures; the accumulation of leaves in damp places, etc.; litter from rabbit-hutches, consisting chiefly of faecal matter, especially at Casa Leoni, where the adult flies were invariably found associated with these animals.

Although one failed to discover either larvae or pupae in any of these situations, it does not prove conclusively, in my opinion, that these insects do not breed in some of them, especially as Grassi[3] has found that in Italy the larvae of *P. papatasii* live in dark damp spots amidst all kinds of refuse in underground places such as cellars, and particularly on the sides of drains which are kept moist by occasional splashes of dirty water.

Other investigators in Malta have met with results similar to my own. Lieut.-Colonel C. Birt, R.A.M.C.[2], who collected the most varied materials, states that he did not succeed in detecting the ova or larvae in any of the samples, 'nor has the adult *P. papatasii* ever hatched out from larvae which might have been hidden in the materials.' Captain Marett[6] has also made extensive search for the larvae and pupae in similar places and in similar materials, and has failed to find a single example of the insect in any of its stages. In so far therefore as our present knowledge is concerned, the only conclusion which can be drawn from the investigations in Malta is that the chief breeding-places of the Papataci flies (*P. papatasii* and *P. perniciosus*) are the crevices between the loose rocks in caves, stone walls, bastions and similar situations.

The task of finding such minute objects as either the larvae or

pupae of these flies is, however, very great; of the two, the larvae are perhaps the more conspicuous, but these have the remarkable habit of flicking themselves from off the surface of the stone or other objects when exposed to light, and in this way numbers may escape detection even under the most practised eye. The pupae are the more difficult to detect, as, apart from their minute size, the colour so exactly harmonises with the colour of the rock to which they are attached that they are rendered almost invisible, and when detected appear only as a naturally formed granular projection on the surface of the stone. In every sense, therefore, they are highly protective forms, and numbers must necessarily escape detection, more especially when artificial light has to be employed in searching for them. Bearing these facts in mind, large quantities of detritus were collected from many and varied sources so that it could be examined under more favourable conditions, but in no single instance were these insects found in either of their preliminary stages, though a lens of low magnification was almost invariably employed in searching for them. Quantities of the detritus were also kept in large vessels in the hope that adult flies might be successfully reared from it; in this again complete failure was the result. As to the detection of the ova in a state of nature I believe this to be a practical impossibility, as when laid upon dark substances they become absolutely invisible and can be detected only by the aid of a microscope. Even when laid in captivity in confined areas they are most difficult to detect, and under the most favourable conditions can be seen only when laid upon colourless or transparent surfaces such as white paper or the surface of a glass tube.

HABITS AND OCCURRENCE OF THE ADULT FLIES

Though so evasive in their early stages, the adult flies may be found almost everywhere throughout the Island in favourable situations or localities. They outnumber the mosquitos, and the females may be included among the most vicious of all the blood-sucking Arthropods. They are distinctly 'domestic' in their habits and may be considered among the most detestable of all man's 'uninvited guests.' It is a curious fact, however, that they

have their likes and dislikes both in regard to hosts and habitats. I can fortunately place myself among the small numbers of those who have proved immune to the bites of these blood-sucking pests; or at least I have never consciously experienced the effect of their bites, any more than I have in the case of *Pulex irritans*. And this is all the more extraordinary because fresh comers to the Island, especially children, generally suffer torture from the bites of these insects, and many cases are admitted to the hospitals through the infection which the Papataci flies are known to convey. To say the least, they are an intolerable nuisance in every part of the world in which they are known to occur. Man is evidently not the only vertebrate which these insects attack, as examples were frequently found which had filled themselves to repletion with the blood of the domesticated rabbit; so that it is evident that they are not entirely dependent upon man for food, and the probabilities are that they subsist and flourish on any of the warm-blooded animals when man is not available.

My experience with regard to the favoured haunts of these flies is almost precisely the same as that of other investigators. In certain parts of the island they were found to be abundant, while in others, for some unaccountable reason, they occurred very sparingly, though the conditions necessary for breeding purposes, especially stone walls, abounded everywhere. In badly infested regions, too, they favoured certain dwellings much more than others; of two houses occupying the same aspect and surroundings, or a section of the same block or street, one was often found to be infested while the other was rarely visited. It was noted also that there was a marked domiciliary distribution in many houses. Bedrooms on the first floor, especially those occupying a position on the lee or sheltered side of the house, were particularly favoured, while those on the opposite side of the building were rarely visited; and rooms at a greater elevation (second floor), which I had under close observation for a considerable time, were only once found to contain a single example.

The naval and military camps at Ghain-Tuffeiha afforded also a remarkable instance of the local distribution of these flies, the naval camp on one side of the plain being badly infested, while the other and more extensive camp was said to be practically free

from the invasion of *Phlebotomus*. This remarkable localisation was in all probability due to the fact that the naval camp was bounded on one side by rocky ground and stone walls, affording excellent breeding-grounds for the flies, while the military camp was remote from such surroundings, and lying fully exposed in the open plain.

At times also, when Papataci flies were literally swarming in houses near the old bastion at Floriana, not a single individual was discoverable in the city of Valetta, half a mile away. In this instance also one may safely infer that the flies at Floriana were breeding in close proximity, and it is highly probable that the actual site was in the interstices between the masonry forming the old fortifications, only a few yards distant from the dwellings.

The daylight retreats of these flies were often similar to those in which they were found at night, providing always that there was an absence of direct light. Thus in the dwelling-houses and barracks, the flies were found at rest in the dark corners of the rooms, under garments, behind pictures and in other similar places; but in nearly all cases they occurred in considerably smaller numbers than at night, though there were one or two noted exceptions. In one instance they could be found in considerable numbers in a badly lighted bedroom at any time of the day, especially after a still, damp night with a heavy sirocco. Odd examples were also found in cellars and in the prison cells in the heart of Valetta; while numbers could be found almost at any time in the small caves or isolated catacombs at Notabile, and such retreats seemed to be one of their favourite haunts during the day. In the early mornings, shortly after daylight, examples of both sexes may frequently be found inside the mosquito curtains, and after favourable nights they sometimes get entrapped in large numbers by this means. On the slightest disturbance the males may readily effect their escape through the meshes of the net; but the females, which are generally engorged with blood, are, under such conditions, much more sluggish than at other times and may then be captured with comparative ease, as they cannot escape through the net very readily when the body is distended with food. In one or two instances Papataci flies were dislodged from the interior of stone walls by forcing tobacco smoke into the interstices; but one met

with such little success that this method was abandoned. Sections of the lower portions of stone walls were also covered with chiffon and carefully examined at intervals during the night, and although the most favourable structures were selected for the purpose, and areas 36 square feet in extent were most carefully covered, not a single fly was entrapped by this method. This is all the more strange seeing that Captain Marett has met with marked success by adopting the plan even on a smaller scale. However this may be, it is perfectly obvious that in the light of Captain Marett's experience stone walls, especially those from which the surface 'pointing' has fallen away in patches, leaving free access to the interior, are the frequent and possibly the principal resorts of the parent flies.

Atmospheric conditions have undoubtedly a marked effect upon the flies. On still sirocco nights they take wing freely and occur in dwellings in larger numbers under such conditions than at any other time. On the other hand, when fresh cool breezes are blowing, especially from the north-west, they are rarely seen; and it is the testimony of everyone who has studied their habits that these insects remain in their hidden and sheltered retreats and rarely venture forth at such times. There is little wonder at this, as their frail bodies and delicate wings are ill-suited for flight under such conditions; moreover, it is a habit common to many members of the same order; minute midges, in particular, are often seen to swarm on still warm evenings, and rarely if ever assemble in numbers under any other circumstances.

A general belief is held by the Maltese that certain kinds of trees and shrubs (fig and loquat especially) form the principal resorts of these insects, and many are also under the impression that they breed either in the foliage or branches or in the fallen and dead leaves which lie beneath them. There may of course be a measure of truth in these theories; but we may at once dismiss the statement that they breed in the trees. It is perfectly obvious, however, that the presence of ornamental shrubs and fruit trees in the walled-in gardens would afford them just the kind of shelter and shade which they require, and would enable them in all probability to travel the more safely from their breeding-places to the house in the immediate vicinity. It is just possible that rotting vegetation in

damp shady places, such as shrubberies, may form a breeding-place also, but so far as our researches have extended up to the present moment we have no evidence in support of this view. Considerable attention was paid to searching such materials but with negative results, as has already been stated. It is clearly evident moreover that dry materials, whether in a state of decay or otherwise, do not form a suitable breeding-place, especially dead leaves which may accumulate on the surface of the ground beneath the trees; light and dryness being both unsuitable conditions for the preliminary stages of the *Phlebotomus*.

The characteristic attitude of *Phlebotomus* is portrayed on Plates VI and VII. When at rest the wings slightly diverge and are elevated at a considerable angle above the thorax and abdomen. On the least disturbance the insects make short rapid flights, almost invariably to the right or left, reminding one of the rapid movements of a flea rather than those of a winged insect. Occasionally, however, they will take long-continued flights, when the course is more or less direct and distinctly midge-like. Their movements on the wing can be followed with little difficulty in daylight, but by artificial light it is almost impossible to do so for more than a few seconds at a time.

Both sexes live but a short time in captivity, unless they are fed upon human blood. Without this they will subsist on wet blotting-paper or other damp materials, such as soil, fresh leaves, etc. Under such conditions many examples survived for periods varying from three to nine days though the majority died on the third and fourth days, even although the females, in many instances, had taken a meal of blood a few hours before they were captured.

SEASONAL PREVALENCE

The adult insects were more or less prevalent during the whole of my stay in the island (July, August, and the first week in September). That the numbers fluctuated during this period has already been mentioned, but this was apparently due, in a large measure at least, to variations in temperature, humidity, and wind. Relatively few Papataci flies occur before the middle of June, and

practically all observers of their habits informed me that they occur most freely and are most troublesome during the hot, dry months of the year. It is highly probable that successive broods are produced during the summer months, but as the larval stage occupies apparently a long period, the successive generations can be produced only at extended intervals.

As to whether the larvae occur most frequently during the summer remains to be seen. It is my impression, however, that they may be found more abundantly in autumn and winter than at any other season, and careful search should be made for them a week or so after the adults have disappeared.

PROPHYLACTIC MEASURES

In consideration of the facts which have so far been brought to light regarding the economy of *Phlebotomus*, it is clearly evident that the task of suppressing these insects is an almost insurmountable one. Had we to deal with insects as large and as accessible as mosquitos, the adoption of prophylactic measures would be comparatively easy, but owing to the extremely minute size and almost flea-like habits of the adult insects, and the enormous area over which the breeding-places may occur, we are faced with a problem which is most difficult of solution.

As I was unable to devote any time to experimental work bearing upon the control of these pests, the only course open to me now is to suggest a few measures which may ameliorate the existing conditions and lead to a reduction of the malady of which these insects are transmitting agents. It seems to me, however, that the only practical way of grappling with this question is to proceed tentatively at first, and although I have discussed an extensive field of operations which may be directed against these insects, I would pin my faith rather to some of those measures which are considered under the following headings. But in the first instance it must be borne in mind that precautions against the bites of blood-sucking insects, though feasible to intelligent and well-to-do persons, are not as a rule employed by the mass of the people. Yet any prophylactic measures which are calculated to diminish the infection, even in a small degree, should be seriously and persistently employed.

Repellents.—I had no opportunity of demonstrating the value of these by experiment owing to my immunity from the bites of these insects, but I was assured that several good formulae were in general use, though proprietary preparations were rarely employed. Judging by the testimony of those who had used such deterrents, one of the best was that which was prescribed by Major Crawford, R.A.M.C., and I am extremely indebted to him for giving me permission to embody it in this report. It is composed of the following ingredients:—

Ol. Anisi, one drachm.

Ol. Eucalypti, one drachm.

Ol. Terebenth, half a drachm.

Unq. Acid Borac, one ounce.

Spraying with repellents.—The least objectionable of these, and at the same time one of the most effective, is formalin. The dark portions and angles of sleeping apartments should be sprayed with a 1 per cent. solution of this substance every day during the season in which the flies are prevalent; a fine spraying apparatus is necessary for its application, and an excessive amount must not be applied. It is considered an excellent plan also to spray the mosquito curtains regularly every day towards sunset; nets thus treated are claimed to repel the attacks of these insects.

Fumigation.—There are several substances which are employed as fumigants for the destruction of insects, but I fail to see the practical utility of employing such means for the destruction of Papataci flies in Malta or elsewhere.

Light.—Daylight is a most important factor in driving away these insects from man's dwelling-places, and directly a flood of light is admitted to a room in which Papataci flies may be present, they immediately seek places of concealment behind garments or draperies and pictures, or other furniture which may be suspended from the walls or placed in dark corners. It is important, therefore, that as much light should be admitted into the rooms as is possible, and this can easily be done either in the early morning or evening, or when the windows are lying in shadow.

Beds should be arranged in the best-lighted portions of the

room, and on no account should children's cots be placed in out-of-the-way corners in deep shadow. Decorative drapery in such apartments should be abolished, and the walls rendered as free from pictures and other furniture as possible.

Artificial light does not, unfortunately, act as a repellent; on the contrary, it would appear to serve as an attraction for these insects, as it is well known to do with other groups belonging to widely different orders.

Artificial air movement.—In India, if not also in other parts of the tropics, it is a recognised fact that punkahs and fans will repel the attacks of mosquitos if continuously and properly employed. It seems to me, therefore, that if a similar method could be applied in Malta, we should be able to dispense with almost every other form of prophylaxis which is discussed in this report. As it has been abundantly proved that Papataci flies do not take wing when the slightest breezes are blowing, one may safely infer that they would not face a strong current of air such as would be produced by either fans or punkahs. It is unlikely that the latter will ever be employed in Malta, but it is my firm belief that if electric fans were fitted so as produce a current of air in the direction of the window in sleeping apartments, that very few, if any, of the flies would be able to pass through the open window into the room beyond. I venture to recommend, therefore, that this method be put to the test, and if found to give satisfactory results, that it be employed in all cases where the cost of running such an apparatus is not a serious consideration.

Traps.—If a modified form of the biscuit-box trap, such as is used for capturing mosquitos, were fixed high up in the dark corners and angles of the rooms, I believe that numbers of Papataci flies would be entrapped. The trap should be made in the form of a corner-cupboard in miniature, and should measure about eighteen inches in length; the basal portion should be left open, and the interior should be lined with dark cloth or similar material. These should be examined daily and the flies killed with ammonia fumes.

Nets.—The use of ordinary mosquito nets is of no avail against the bites of these pests, as they readily pass through the meshes, and attack persons just as freely as if nets were not used; but if they could be rendered repulsive to the insects by spraying them

with formol or other repellents, as has been suggested, so much the better; but experiments in this direction must be conducted before we can say definitely that such a method would prove effectual. Fine nets made of strong chiffon or other similar material would undoubtedly prevent the approach of these flies, but the use of such nets would render sleeping almost impossible in the hot weather unless electric fans were used at the same time. If such preventive measures as these could be employed to the complete satisfaction and comfort of patients in hospitals, especially those suffering from the Papataci fever, or to the community in general we shall have succeeded in devising an excellent prophylactic measure. If a net of this type is used, it should have a strip of calico about two and a half feet in width stitched all round the bottom, so that at least twelve inches of it extends above the bedding, the remainder to be tucked in under the mattress. The use of this is obvious; the strip above the bedding would prevent the flies from biting any portion of the body which might be brought into contact with it, and the lower portion of it would stand the strain of 'tucking-in,' and consequently last for a very much longer time than such flimsy material as chiffon.

Destruction of breeding-grounds. — As to the operations necessary for reducing the number of breeding-places, it is perfectly obvious that we can never expect to be able to deal with these in any of the rural districts, owing to the fact that the fields and roads extending over the whole of the country are bounded by stone walls, and elsewhere there are fissured rocks, caves, and other suitable places which afford just the right conditions necessary for the breeding of Papataci flies. On the other hand, we may reasonably hope to reduce them in the principal centres of population, if persistent efforts are made to accomplish this, and if financial considerations do not prohibit the employment of such methods as are herein suggested. If it should be considered advisable to carry out any section of this part of the propaganda, one of the smallest and most isolated of the infested areas should be chosen as an experimental ground, and an officer who is thoroughly acquainted with the habits of the insects should be appointed to direct the operations. If loose rubble walls exist in the immediate neighbourhood of the selected area, these should be

either demolished and the materials removed, or they should be completely covered with a thick layer of cement.

If such a type of wall exists as has the jointings partly filled with plaster ('pozzolani'), then all openings and fissures should be carefully filled in with cement, so that no holes are left for the ingress or egress of the flies, remembering always that a crevice sufficiently large to admit a flea will also afford ample space for the admission of the fly.

If it should be found necessary to replace the old walls with new ones, it is imperative that these should be built of solid masonry to a height of at least two feet above the level of the soil on either side, as it is the lower portions of the walls that are, according to Captain Marett's experience, selected as breeding-places; but it would be better, in my opinion, to make all new walls of solid masonry from the foundation to the topmost course or layer; and if the old walls could be substituted by any other form of boundary, so much the better.

There are also other kinds of walls which may have to be dealt with, and these are they which form the old bastions and other extensive fortifications at Cottonera and elsewhere. In cases where such structures are backed with rubble and finally protected with loose rock, it would be a comparatively easy task to prevent the egress of the flies through such loose material by breaking or pulverising it, or by covering it with soil; but unfortunately the question of pointing the Ashlar work forming the facings of the bastions and curtains presents not only a serious financial difficulty, but a task which could be accomplished only by a huge army of men; and in consideration of these facts it seems to me that in the present stage of our inquiry such a method of procedure would be extremely unwise and irrational. For the time being, therefore, I should strongly advise that in selecting the experimental area a site should be chosen which is as remote from the old fortifications or similar structures as is possible.

Though there is no evidence which will lead us to believe that Papataci flies breed in the cellars and drains in Malta, at the same time we must not lose sight of the fact that Grassi[3], as has already been stated, has found larvae of *P. papatasii* in such places. It is highly probable, therefore, that this species breeds in similar

habitats in Malta also; but it is impossible without more study to make any definite statement on the point. Taking all the facts into consideration, therefore, I consider that the only really practical prophylactic measures which can at present be taken are those which are considered as precautionary against the bites of these insects. It is perfectly obvious, moreover, that any operations which will not bring about an almost complete destruction of the breeding-grounds are not likely to make an appreciable reduction in the numbers of these insects.

SYNONOMY, AFFINITIES, AND MORPHOLOGY OF THE GENUS PHLEBOTOMUS

Though the differential characters of this genus have been given by several authors, and Grassi[3] has published an elaborate memoir on the morphology and biology of *Phlebotomus papatasii*, I consider that this report would be incomplete without giving some details concerning the morphology of these insects; all the more so because Grassi's paper, in Italian, is now very difficult to obtain and also a very costly publication, in fact the price (£1 10s.) for so small a work, is practically prohibitive, and certainly not within the reach of students in general.

I do not claim, however, to treat of this phase of the subject in an exhaustive way, but rather to point out the salient characters of these insects in a measure that may be helpful both to the medical profession and to the zoologist.

The genus *Phlebotomus* was established by Rondani in 1840, though the species for which it was founded had been placed by various authorities in other genera, such as *Bibio* (Scopoli, 1786), *Musca* (Gmelin, 1788-1793), *Ciniphes* (Costa, 1840). But as Rondani's name is now generally accepted, one need not go into further details regarding the nomenclature and synonyms of *Phlebotomus*. The taxonomic position of this genus is with the family PSYCHODIDAE, and it is included in the sub-family PHLEBOTOMINAE. All the members of this family are small Nemocerous insects characterised by the possession of relatively large wings which are clothed with either scales or hairs; and one of the most familiar representatives, and one also which is widely

distributed and nearly related to *Phlebotomus*, is the genus *Psychoda* (sub-family PSYCHODINAE) the members of which are known generally to Englishmen as 'Moth-flies' or 'Owl-midges.' The short diagnosis which follows will serve at once to distinguish *Phlebotomus* from any of the allied genera in the PHLEBOTOMINAE and also from the midges belonging to the PSYCHODINAE.

Genus PHLEBOTOMUS, Rondani

Mouth formed for piercing and sucking; palpi of five segments; antennae long, filiform and composed normally of sixteen segments; wings hairy, narrow, second longitudinal vein twice forked, cross-veins placed near the basal fourth of the wing; body clothed with hairs; sexual dimorphism distinct.

The larva (Pl. V, figs. 7-8) is characterised by its caterpillar-like form (eruciform); by the presence of two pairs of long caudal bristles, which may equal the length of the body; and by the absence of true legs.

The pupa (Pl. V, fig. 12) is obtectate, and may be recognised by the presence of the larval skin which invariably remains attached to the last two segments of the abdomen. It should be borne in mind, however, that the partial retention of the larval skin by the pupa, is not peculiar to the genus *Phlebotomus*, as Speiser[8] has shown that the larval skin of *Helea* (*Forcipomyia*) *regulus*, Winn., one of the members of the CHIRONOMIDAE, also remains attached to the anal segments of the pupa. The larva of this genus does not, however, possess the long caudal bristles which are so characteristic of *Phlebotomus*, though in other ways it is not unlike the latter.

EXTERNAL MORPHOLOGY

Head (figs. 1 and 9) somewhat elongated, but distinctly narrowed at the nape, vertex clothed with long hairs; clypeus large and also clothed with hairs on the upper surface. Eyes large and intensely black.

Antennae (fig. 2) very long and slender, and in all of the Maltese species consisting of sixteen segments; the first and second segments forming the scape are short and stout, the second one

being somewhat spheroid in shape; the third is much the longest and of uniform width throughout; the remaining segments are gradually swollen proximally, especially the terminal ones; all are clothed with hairs; those arising from the swollen portions being much the longest and considerably longer than the individual segment to which they are attached. In all of the Maltese species there are also present on several of the segments, and in both sexes, *a pair of relatively large geniculated spines* (fig. 2). These curious

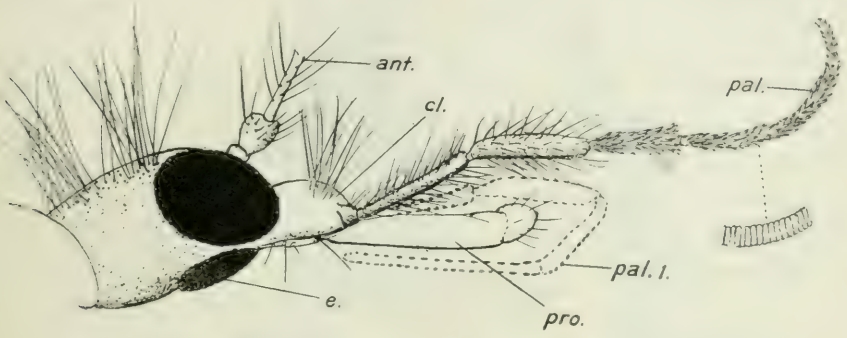


FIG. 1. --Head of *Phlebotomus papatasii*; *ant*, antenna; *e*, eye; *cl*, clypeus; *pal*, palpus; *pro*, proboscis.

appendages are rendered practically invisible when the segment to which they are attached is mounted so that a dorso-ventral aspect is presented under the lens of the microscope; and for this reason apparently they have been hitherto overlooked by all the students of this genus of insects. It is true that Grassi[3] (p. 12) has noted that 'here and there one can observe a short hair curved and relatively thick'; but that he failed to recognise the true character and arrangement of these spines is perfectly clear. Now that they have been discovered it is highly probable that they will be found to exist in the majority of species, if not in all, and may I think be considered of generic importance. Annandale, in his description of the genus *Brunettia*, a new Psychodid discovered in Southern India, refers to a similar character, but in this instance the paired spines are somewhat **S**-shaped and relatively much stouter than the corresponding spines in *Phlebotomus*. In the light of these discoveries, therefore, it is possible that similar spines may be discovered in various other members of the same family, though

it is highly improbable that such structures will eventually be found to exist in all of them.

Palpi (figs. 1 and 13).—These organs are generally said to be composed of four segments, but there are undoubtedly five, and



FIG. 2.—Antenna of *Phlebotomus papatasii*.

this number may, I think, be considered common to all the members of this genus. Annandale[1] has pointed out that 'a minute basal

joint can sometimes be distinguished in fresh specimens,' but that it is 'often difficult to see and appears to be imperfectly separated from the others.' That the small basal segment is clearly articulated to the second there can be no doubt, as it can be seen quite distinctly when mounted so that it is not obscured by the surrounding structures. All of the segments are clothed (in *P. papatasii* at least) with variously formed scales, intermixed with a few hairs. The scales on the first three segments are for the most part very long and somewhat hair-like, those on the remaining segments short and closely packed together. The fourth and fifth segments, especially the latter, are distinctly but somewhat irregularly annulated or ringed, a character which has also been hitherto overlooked by former investigators. In life, when these organs are at rest they are bent downwards and backwards at the articulation of the third and fourth segments, so that the anterior half of the palpus is folded back more or less upon the proximal half; by this curious arrangement practically the whole of the proboscis is covered or protected (fig. 1, *pal.* 1).

Proboscis (figs. 1 and 3).—Slightly shorter than the head, inclusive of the clypeus; in form it is somewhat cylindrical and slightly recurved distally. In the female it is composed of the following parts:—*The labium* (fig. 3, *lb*). This is much the largest organ, and as far as one can judge by viewing it in optical section, it almost completely embraces the labrum-epipharynx; the proximal half is sparsely clothed with lanceolate scales, and the first third is markedly narrower than the rest; immediately in front of the dark chitinous apodeme or sclerite is a curved row of long fine hairs; the labella are scarcely broader than the widest portion in the region of the apodeme, and are clothed with a number of fine and rather long hairs. *The labrum-epipharynx* (fig. 3, *lbr*) is relatively narrow and the sides are parallel, but the apex is suddenly attenuated the tip bluntly pointed, and the margins furnished with a series of long spinose teeth set closely together and numbering about twenty on either side; ventrally it is deeply and broadly channelled but does not appear to possess interlocking teeth or other structures. *The hypopharynx* (fig. 3, *hy*) is similar in width and general form to the labrum, but tapers off much more gradually towards the end, and the marginal

spinose teeth are much shorter and placed so closely together as to present a finely serrated edge; its upper surface is distinctly and broadly concave or trough-like and the salivary duct which is small, occupies a central position. *The mandibles* (fig. 3, *md*) are broad and blade-like, and have the outer edges faintly serrated, the

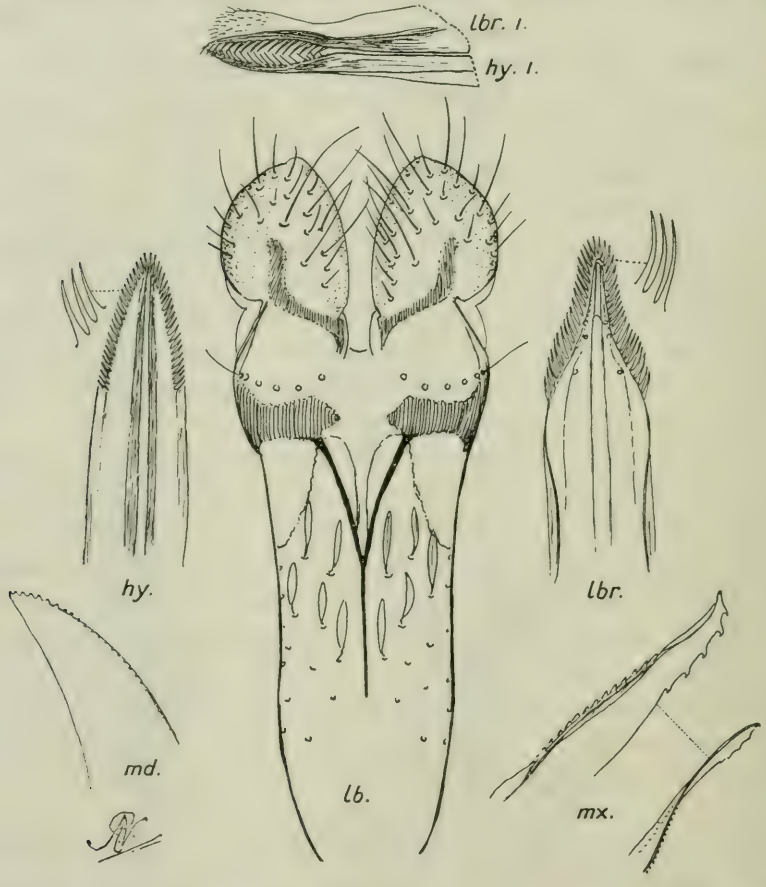


FIG. 3.—Mouth-parts of *Phlebotomus papatasii*, ♀; *lb*, labium; *lbr*, labrum-epipharynx; *hy*, hypopharynx; *md*, mandible; *mx*, maxilla.

The upper figure represents the labrum *(lbr* 1) and hypopharynx *(hy* 1); male, *P. papatasii*, as seen in profile.

serrations being rather widely separated. When at rest they lie, apparently, superimposed one over the other. *The maxillae* (fig. 3, *mx*) are much narrower than the mandibles, curved transversely, and attached to a broad trough-shaped sclerite, not to

a long slender stalk as Grassi has shown. One edge is provided with five relatively large and widely separated teeth; the opposite edge with smaller ones set closely together.

Thorax (fig. 8).—This consists largely of the meso-thoracic division, the prothorax being represented by a very short extension which can be seen more or less distinctly in examples which have been macerated and mounted in Canada balsam. The scutellum and post-scutellum are well developed and conspicuous in mounted preparations.

Abdomen.—This is composed of ten segments, the last being modified by the external genitalia. In the female the appendages are simple, flattened, leaf-like structures, densely clothed with hairs and arranged in two pairs (figs. 8 and 10). In the Maltese species they are all so similar in structure as to afford no diagnostic characters of importance. Annandale[1] states (p. 41) that these organs 'become distorted and shrivelled in dried specimens.' These structures can, however, be restored by maceration in caustic potash but the best results may be obtained by preserving the specimens in alcohol.

External genitalia of the males.—These are large and complex structures (figs. 14-18) and afford a ready means of determining the sexes, moreover, their morphological characters are of great importance as they present very marked specific differences whereby the closely allied species may be readily distinguished. These appendages are arranged in five pairs as follows:—*Superior claspers* (*sc* in all the figures). These are placed dorsally and are larger than any of the other structures; they are composed of two distinct segments, of which the terminal or distal one is the smaller and is provided at the apex with large spines, which in some species are curiously modified. They are generally densely hairy and large scales may also be present; but both hairs and scales are easily deciduous and the greater portion of them usually fall away during the process of mounting for microscopical study. The accompanying illustrations must therefore be considered as representing these structures in a partly denuded condition. *Inferior claspers* (*ic*). These are unisegmented and much shorter than the superior pair; they are ventrally placed and may or may not have modified spines at the distal extremity. *Submedian*

lamellae These lie between the inferior claspers, and although they are usually short, thin, leaf-like structures, in some instances (*P. minutus*) they are very similar to the clasper both in form and length. *Intermediate appendages* (*ia*). These occupy a median position and are often curiously modified; they form a branch of the superior clasper and are sometimes bi-lobed. *Intromittent organ* (*io*). This is homologous with the 'juxta' in *Glossina*, and is described as the penis by Grassi. It consists of a pair of long slender and highly chitinised organs which lie between the intermediate appendages. These completely ensheath the two long filamentous processes which form a continuation of the ejaculatory duct leading from the penultimate segment of the abdomen. In *P. papatasii* they have not been seen to extend beyond the intromittent organ or penis, while in *P. perniciosus* (figs. 16, 17), though lying apparently in a normal resting position, they project beyond it to a distance equalling one-half the length of the sheath.

Wing.—This is densely hairy, and may at once be distinguished from that of the mosquitos (CULICIDAE) by the entire absence of scales, the double fork of the second longitudinal vein, and the proximal position of the cross-veins. The hairy character is well shown in the illustrations (Pl. VI, fig. 2, and Pl. VII, figs. 1, 2), and when denuded (figs. 4-7), the venation can be seen with little difficulty in properly prepared specimens. The costa is the thickest of the veins. The sub-costa, in comparison with that of the CULICIDAE, is very short, curves downward distally, and joins the first longitudinal vein at or about one-fourth of the distance between the base and tip of the wing. The first longitudinal is simple, and unites with the costa about one-third from the tip; the second longitudinal is twice forked, and extends almost to the base of the wing; the third longitudinal is simple, and originates from the mid cross-vein; the fourth has origin at the base of the wing and is forked near the middle; the fifth and sixth are simple and united basally, the former curving upwards and uniting with the fourth considerably in advance of the base of the wing. The first cross-vein unites the costa with the sub-costa at a point immediately opposite to the turned-down portion of the latter, so that in effect they produce two cross-veins: the first



FIG. 4.—Wing-venation of *Phlebotomus papatasii*; upper, ♀; lower, ♂.

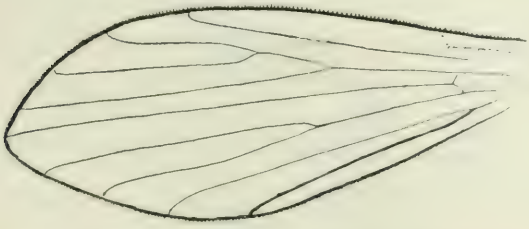


FIG. 5.—Wing-venation of *Phlebotomus nigerrimus*.

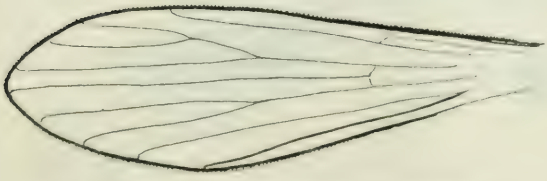


FIG. 6.—Wing-venation of *Phlebotomus perniciosus*.

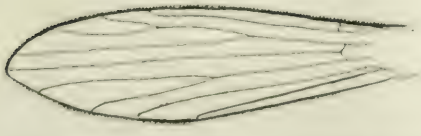


FIG. 7.—Wing-venation of *Phlebotomus minutus*.

extending from the first longitudinal vein to the sub-costa, the second from the tip of the latter to the costa. The mid cross-vein arises from the base of the third longitudinal and passes obliquely to the fourth; while the supernumerary vein is placed immediately above it, and passes obliquely to the second longitudinal.

Legs.—These are very long and slender and densely clothed with scales, the majority of which are flat and closely resemble those which are found in the CULICIDAE. The ungues are simple in all of the Maltese species, and do not offer any differential morphological characters.

INTERNAL MORPHOLOGY

The Alimentary Canal (fig. 8).

This structure differs from that of the mosquito in having a true sucking stomach, and also in the possession of four malpighian tubules instead of five. The general form and relative position of these organs in the female are as follows:—

The buccal cavity lies at the base of the clypeus; it is dilated distally, but almost immediately contracts and forms a slender tube which leads to the pharynx.

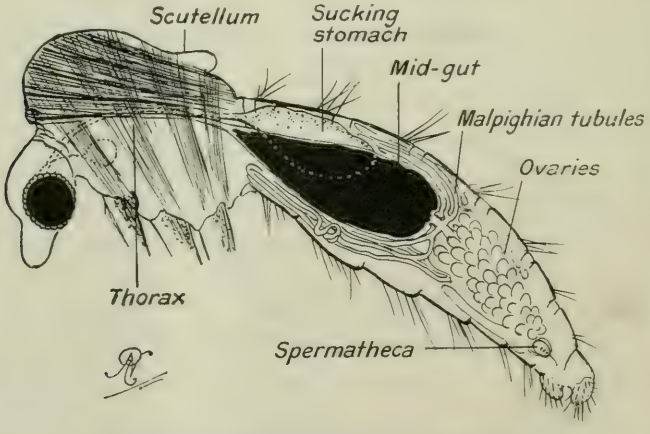


FIG. 8.—Internal morphology of *Phlebotomus*.

The oesophagus divides at a point a little in advance of the posterior margin of the head (nape), one tube leading to the sucking stomach, or food-reservoir, the other to the digestive canal.

The sucking stomach.—This is a large, thin-walled pouch, connected with the end of the oesophagus by means of a very slender tube. It lies on the left side of the digestive canal, and extends distally as far as the region of the fourth abdominal segment.

The mid-gut or chyle stomach.—This is capable of great distention, and when filled with fresh blood occupies a large portion of the abdominal cavity; but when such food has been partly comminuted it becomes much smaller, and can be easily seen as a black, elongated pouch in the anterior portion of the body.

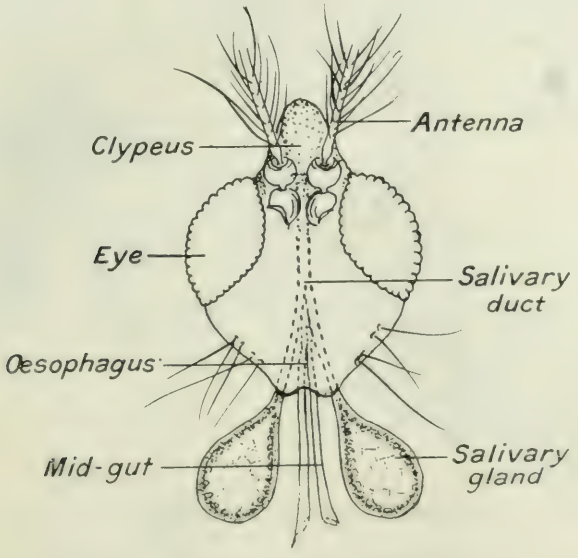


FIG. 9.—Head of *Phlebotomus*, showing position of salivary glands.

Malpighian tubules.—There are two pairs of urinary organs, each pair being united at their bases, where they form a single tube, which is connected with the intestine immediately below the mid-gut. They are of great length, extending forwards as far as the first abdominal segment, where they are folded and doubled backwards upon themselves, and also form loops in the mid-region of the ventral portion of the abdominal cavity.

The salivary glands (fig. 9).—These consist of two broadly dilated or lobe-like acinous glands, lying one upon either side of the prothorax. The periphery of these glands presents an even or

smooth surface, and immediately within the exterior wall is a series of rather large secretory cells. The ducts leading from the acini unite near the mid-region of the head, forming a common duct, which enters the buccal cavity close to the base of the clypeus.

The Sexual Organs of the Female.

The ovaries occupy a variable position in the different stages of their development. In the early adult stages of the insect (fig. 8) they are very small, and are seen to extend from just behind the origin of the Malpighian tubules to the region of the penultimate segment of the abdomen. When fully matured (fig. 10) they occupy practically the whole of the abdominal cavity, extending forwards



FIG. 10.—A female *Phlebotomus*, showing fully matured ovaries.

as far as the second segment. They are bi-lateral, and each ovary comprises 20-25 ova, representing a full complement of 40-50, so that these insects cannot be considered very prolific. The tubular oviducts unite at a point just before reaching the base of the inferior claspers, where they form the common oviduct.

The spermathecae (fig. 11) lie in the median line in the region of the oviducts. They consist of a single thin-walled, sub-spherical sac, and are relatively very large; at their junction with the duct they are strongly chitinised, and consist of usually ten transverse and convex ridges, which are so constricted at the margins as to present, in optical section, a distinct and well-marked crenulation. The tubular ducts, which are long and straight, open into the oviduct near to its termination, apparently.

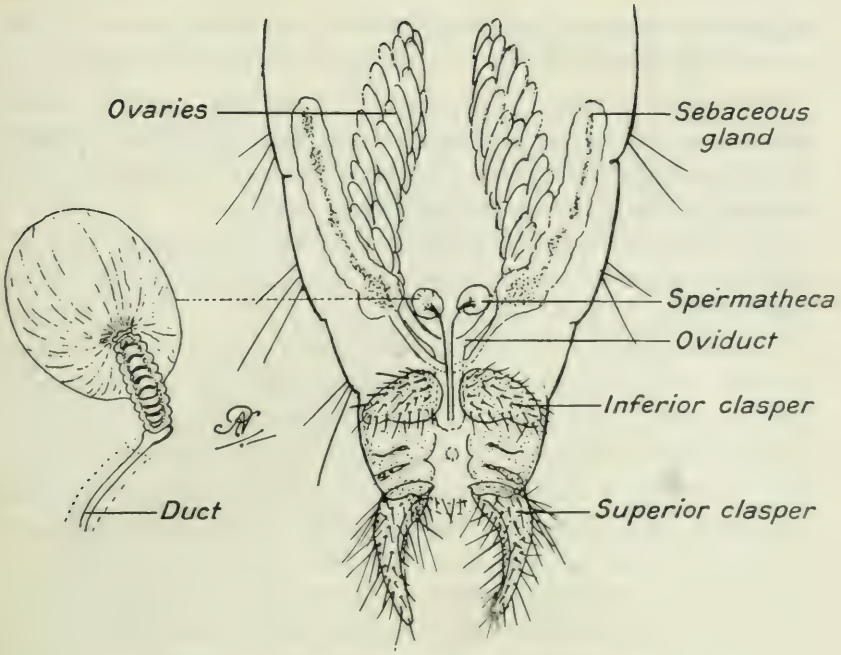


FIG. 11.—Female generative organs of *Phlebotomus*.

Sexual Organs of the Male (fig. 12).

The external characters of the male armature or copulatory apparatus have already been discussed (p. 159) and although the internal sexual organs have been but briefly studied, from

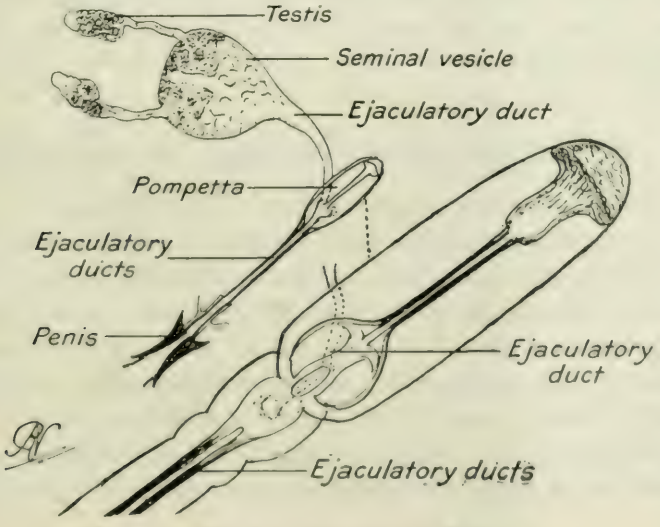


FIG. 12.—Male generative organs of *Phlebotomus*.

preparations examined in optical section, yet a brief account of them may not be without interest. These consist of the following:—

The testes.—These may present a somewhat variable outline, though normally they are elongate-ovate; they are distinctly paired and widely separated, each possessing its own duct which enters the seminal vesicle at its anterior lateral margin.

The seminal vesicle consists of a large pyriform sac, the proximal portion of which gradually narrows and merges into the short, tubular, ejaculatory duct.

The ejaculatory duct is connected with a singular morphological structure which, together with the chitinous rods, presents a slender and somewhat club-shaped though cylindrical process. The outer wall of the swollen portion is formed of thin transparent chitin; and occupying a central position is a piston-like rod which is dilated at both extremities; the distal portion somewhat resembles an inverted bulb, the opposite dilatation forming a more or less spherical sac. The space between the central structures is seen to contain delicate muscular fibres; and the ejaculatory duct leads into the spherical cavity at the lower end of the piston-like rod. Grassi's interpretation of the mechanism of this structure is that it acts like a little pump (*pompetta*) and regulates the exit of the spermatozoa. Beyond this structure the ejaculatory duct is protected by two slender hair-like rods which are highly chitinised and form the 'intromittent organ' which has already been described (p. 160) as extending into and in some cases considerably beyond the penis-sheath or juxta.

OVIPOSITION OF PHLEBOTOMUS IN CAPTIVITY

The act of oviposition was observed on several occasions and was not without interest, as the insect assumed a position which seemed altogether unique and extraordinary. In the first instance, a female with ripe ova was placed in a small glass-topped box, the bottom of which was within focal distance of a lens magnifying eight diameters. She was supplied with blotting paper which had been soaked in clean water. On placing this in the box the insect immediately alighted upon it, brought her proboscis into contact with the paper, and after a few seconds appeared to be perfectly

intoxicated and helpless. Unfortunately she struggled away and was finally hidden beneath the paper so that further observations at the time were impossible. After an interval of a few minutes she reappeared, crawled up the side of the box, and one and a half hours later seemed as active as when first captured. On the following day, at 9.30 a.m., a fresh supply of wet blotting paper was placed in her cage when in less than sixty seconds she alighted upon it and assumed the same extraordinary attitude as on the previous evening at 6 p.m., collapsing immediately and placing her legs so that the middle and hind pair were crossed behind the abdomen, the front pair remaining almost in a normal position. The abdomen was then elevated and extended to the full and three eggs were laid at short intervals. Each egg appeared under the lens as a tiny translucent drop of fluid and was ejected with considerable force to a distance equal to about three times that of the length of the abdomen. This process lasted for about two minutes, and afterwards the female crawled slowly away and up the side of the box, appearing weak and fatigued. Here she remained almost motionless for nearly three hours, gradually raising the whole of the body until it assumed a normal resting attitude.

On removing the blotting paper which had been placed in the cage the previous evening, seven additional eggs were found and these were evidently laid the previous evening when the insect was observed to go through the evolutions which have just been described. At 12.30 a.m. the same day she repeated the process when freshly moistened blotting paper was supplied. On this occasion two eggs were laid and these were found attached together side by side. At 5 p.m. two additional eggs were laid, the same curious attitude being assumed as before, but although frequently supplied with fresh wet blotting paper she did not produce any more eggs, and at 10 p.m. she died. On making an examination of the abdomen it was found to contain eight fully developed ova so that it is quite evident that this female had laid eggs elsewhere and previously to her capture.

The act of oviposition was seen on subsequent occasions, but in two instances the females died after remaining in a collapsed condition for periods of two and a half hours, and three hours and three-quarters, respectively. Both examples had their abdomens

well filled with ripe ova and had apparently not laid any eggs before they were captured.

SYNOPSIS OF MALTESE SPECIES OF PHLEBOTOMUS

A. Abdominal hairs recumbent.

(*a*) Integument black. Large species. Palpi with second segment slightly longer than the third.

nigerrimus, n.sp., p. 168.

(*b*) Integument ochreous. Small species. Palpi with second segment one half the length of the third.

minutus, Rond., p. 169.

B. Abdominal hairs more or less erect.

(*a*) Legs in both sexes relatively short; average length of hind leg, 3 mm. Terminal segment of superior clasper of male scarcely half as long as the inferior clasper.

perniciosus, n.sp., p. 172.

(*b*) Legs in both sexes relatively long; average length of hind leg, 4 mm. Terminal segment of superior clasper of male slightly longer than the inferior clasper.

papatasii, Scop., p. 174.

PHLEBOTOMUS NIGERRIMUS, n. sp.

FEMALE.—*Colour*. Head, thorax, and abdomen brownish black; hairs bright ochreous buff, those on the thorax being slightly paler and erect, those on the abdomen recumbent. Basal segment of antennae dark brown. Palpi pale to dark brown, hairs similar in colour to those on the body. Legs pale ochreous buff, with ochreous white, *not silvery white*, refulgence. Wings ochreous buff or dull golden in some lights.

Head. Proboscis long; eyes black, deeply emarginate in front. *Palpi* and *antennae* very like those of *P. papatasii*. *Legs* very long, femur of hind pair nearly as long as the abdomen; tibia one and one-third times the length of the femur; tarsi longer than the tibiae by about one-sixth, or nearly as long as the wing; ungues simple. *Wings* (fig. 5) with the hind margin strongly arched; sixth longitudinal vein short, terminating near the centre of the hind margin, the length equal to the distance, in a straight line, from its tip to the tip of the third longitudinal vein; the anterior

branch of the second longitudinal vein twice the length of the distance between the two forks.

Length 2·50 mm.

The black or brownish-black colour of the integument of this insect will serve as a ready means of distinguishing it from any of its allies. It may also be separated from *P. papatasii*, to which it is closely related in its morphological characters, by the shape of the wing and the shorter sixth longitudinal vein. The only two examples which were secured were taken by Captain P. J. Marett; both are females, one of which bears the data: 'Black species, Gozo, 20, X, 10'; the other '*P. papatasii*, dark variety, 17, VI, 10, F.'

Captain Marett had evidently, therefore, noted the black or dark colour of this insect in life; and when questioned regarding this he was absolutely certain that the colour was not due to *post mortem* changes. It is undoubtedly a rare insect in the Maltese Islands, otherwise more specimens would have been secured. We trust that Captain Marett will be able to obtain examples of the males so that the characters of the armature may be examined and described.

PHLEBOTOMUS MINUTUS, Rondani.

MALE.—*Colour.* Integument rather opaque, dull golden ochreous. Antennae with black and ochreous hairs mixed. Head with the clypeal and occipital tufts of hairs pale ochreous. Thorax with a median mane-like tuft, a lateral tuft in front of the insertion of the wings and also a tuft on the scutellum, all pale ochreous with a golden tinge, but with a few intermingled black hairs. Abdomen densely clothed with recumbent, dull, golden ochreous hairs; those covering the genital organs intermingled with black hairs. Legs covered with scales which appear smoky brown in some lights, silvery ochreous in others. Hairs of the wing mixed black and ochreous, those of the costa not darker than those on the surface of the wing.

Head. Proboscis relatively short; clypeus hairy. *Antennae* with the third segment a little longer than the fourth, but not nearly so long as the fourth and fifth together; the long verticillate hairs extending to the apical segment. *Palpi* (fig. 13) with the

second segment one-half the length of the third; the latter much the stoutest and broadest; dorsally it appears incrassate towards the base; fourth segment not quite so long as the third; fifth much the longest.

Wings (fig. 7) very narrow, and bluntly lanceolate; divided into two almost equal halves by the third longitudinal vein; the upper or anterior branch of the second vein shorter than the distance between the two forks.

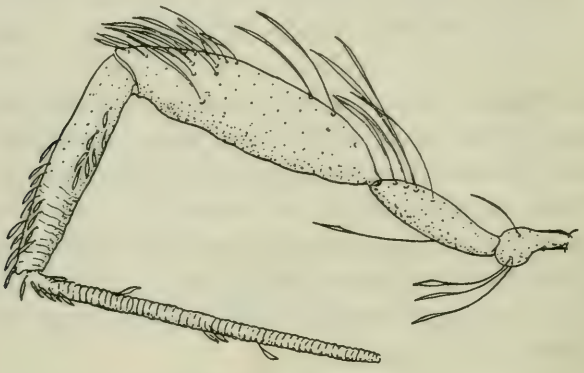


FIG. 13.—Palpus of *Phlebotomus minutus*.

Legs. Hind pair a little more than three times the length of the abdomen inclusive of the genitalia; tarsus a little longer than the tibia.

External genitalia (figs. 14, 15) small; superior claspers with four long spines: two apical and two subapical: inferior claspers very slightly swollen in the middle; intermediate appendage similar to that in *P. papatasii*; intromittent organ nearly three-fourths the length of the inferior claspers; genital filament not protruding.

Length 1·5-1·65 mm.

FEMALE.—*Colour*. Wings with a distinct black costa and fringe; wing-area also with numerous black hairs intermixed with the ochreous ones. Legs with the femora ochreous beneath, darker above; tibiae and tarsi blackish, with silvery grey scales. Thoracic and abdominal hairs as in the male.

Antennae with the long hairs extending to the tip, the third to

the ninth segments, inclusive, with geniculated and paired spines. *Palpi* as in the male.

Length 2 mm.

The distinguishing characters of this insect are its relatively small size, especially in the male; the recumbent abdominal hairs; the short third antennal segment; and the marked character of the palpi. The male may be easily distinguished also by the form of the external genitalia.

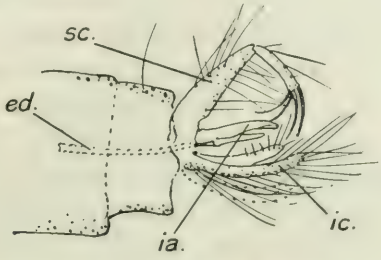


FIG. 14.—External genitalia of *Phlebotomus minutus*, ♂; *sc*, superior claspers; *ic*, inferior claspers; *ia*, intermediate appendages; *ed*, ejaculatory duct.

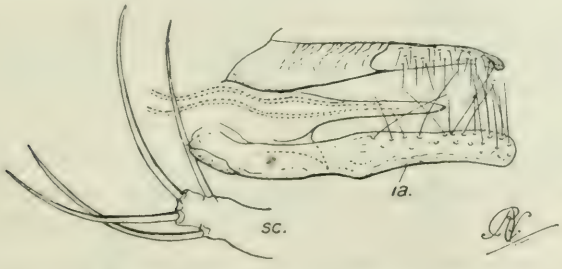


FIG. 15.—Superior clasper (*sc*) and intermediate appendages (*ia*) of *Phlebotomus minutus*, more highly magnified.

The first two examples were captured by Major F. L. Dibblee, Royal Marine Artillery, at his residence at Sliema, August 20th, 1910; and two additional specimens were taken by myself, one at Casa Leoni, in a rabbit hutch, August 31st; the other at Floriana, August 27th.

In captivity *Ph. minutus* is much more active than any of the other Maltese species, and when confined to a small area was almost incessantly moving from place to place. Apart from its flea-like actions it also has the remarkable habit of whirling round and round with great rapidity, so rapidly at times as to render itself almost invisible.

PHLEBOTOMUS PERNICIOSUS, n. sp.

MALE.—*Colour immediately after death.* Eyes black. Thorax with or without dull red-brown spots; when present they are arranged in a triangle, and there is occasionally a similar spot on the vertex of the head. Thorax and coxae pale, translucent ochreous; abdomen similar, but sometimes pale smoky grey. Hairs pallid. Wings faintly iridescent in strong light; pale drab in subdued light; costal fringe generally very dark or blackish grey, though examples with pale costal fringes are not uncommon. Legs silvery grey, in a strong light presenting a distinct metallic lustre; in certain lights also those segments which lie in shadow appear almost black and show up in marked contrast to those which are so placed that their surfaces refract the light. In some lights the under surface of the legs appears distinctly and regularly speckled, a character due evidently to the regular arrangement of the scales.

Head densely hairy, with generally two ill-defined tufts. Clypeus with a large tuft of hairs, some of which are directed forwards, others backwards towards the forehead.

Palpi with segments 2, 3 and 4 equal in length and collectively a little longer than the 5th. *Antennae* with the second segment much longer than the two succeeding ones; the longest hairs on segment 14 almost equal in length to those on the preceding segment. *Thorax* densely hairy, usually with a tuft on the front portion and another on the scutellum. *Abdomen* densely hairy, the longest hairs arising from the apical margin of the segments but no distinct tufts are found as in *P. papatasii*. The arrangement of the hairs is similar in both sexes, but blackish hairs are often intermixed with the pale ochreous ones on various parts of the body in the darker forms of this insect. *Legs* shorter than those of *P. papatasii*. *Wings* (fig. 6) with the posterior border much more strongly arched than the anterior border; the anterior branch of second longitudinal vein nearly as long as the stem between the cross vein and the proximal fork.

External genitalia (figs. 16, 17). Superior clasper with five very long stout curved spines; two apical, one external and two internal, placed a little in advance of the outer one; inferior clasper nearly twice the length of the intermediate appendage and clothed to the apex with very long and slender hairs; intermediate appendage

somewhat finger-shaped and hairy, proximal portion with a large keel-like extension ventrally, the distal margin of which bears several (5-6) hairs; apex of intromittent organ deeply divided or forked, with occasionally a minute central tooth; exposed portion of the genital filament about half the length of the intromittent organ.

FEMALE.—With the *palpi, antennae and legs* similar to those of the male. *Wings* very slightly larger and broader than those of the male.

Length 1·9-2·2 mm.

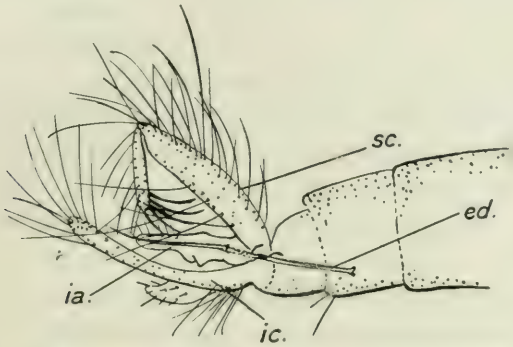


FIG. 16.—External genitalia of *Phlebotomus perniciosus*, ♂ ; *sc*, superior claspers; *ic*, inferior claspers; *ia*, intermediate appendages; *ed*, ejaculatory duct.

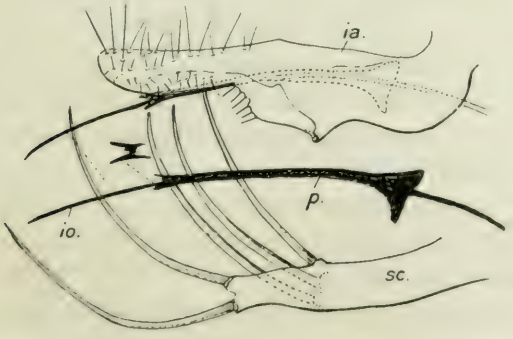


FIG. 17.—Portion of genitalia of *P. perniciosus*, ♂, more highly magnified; *sc*, superior claspers; *ia*, intermediate appendage; *p*, penis; *io*, intromittent organ.

This insect is widely distributed over the island of Malta, and was extremely abundant during the month of August and the beginning of September, though many examples were captured also in July. It was most abundant at Floriana, near the old bastion by the Grand Harbour, on the evenings of August the 26th and 27th,

when, between the hours of 8.30 p.m. and 9.30 p.m., thirty-nine examples were captured as they came into a lighted room; of this total twenty-eight were males and eleven females.

Two examples of *P. minutus* were found in association with this species; but strange as it may seem, not a single example of *P. papatasii* was either captured or seen on these occasions.

It was common also during the last week in August at Casa Leoni, the residence of the Hon. E. C. Roupell, D.S.O., Acting Lieutenant-Governor. In this place it was found most abundantly in a large outhouse which was tenanted by a number of rabbits. In the early mornings, shortly after 6 a.m., numbers of sand-flies were found chiefly in the corners of the room, but many were also seen sitting about the walls in various places, though chiefly at the junction with the ceiling. Later in the day they were rarely seen in these situations; but examples could always be found in the dark earthen pots which were used, and generally occupied by the rabbits as retreats.

The male is easily distinguished from that of *P. papatasii* by its generally smaller size, shorter legs, and much smaller genital armature, which is little more than half the width of the abdomen. The female may also be distinguished by its shorter legs, and generally darker colour. After a few hours in captivity it also becomes generally much less active than *P. papatasii*, though it has the same hopping flight so characteristic of these insects.

Pupa (Pl. VI, fig. 4).—Abdomen distinctly and sharply curved upwards so that a somewhat **S**-shaped outline is produced; thorax gibbose; abdominal segments each provided with a *pair of very large tubercles* (Pl. VI, fig. 5), the tips of which are furnished with a pair of broad flat appendages; integument thickly covered with squamose spines (Pl. VI, fig. 4).

The larval skin attached to the pupa does not present any morphological differences from that of *P. papatasii*, as far as one can gather from its shrivelled condition. It possesses the same kind of caudal bristles and hairy body-spines.

PHLEBOTOMUS PAPATASII (Scopoli)

Bibio papatasii, Scopoli. Deliciae faun. et flor. Insubriciae, I, p. 55, Pl. XXII, fig. B. a. b. (1786).

Cyniphes molestus, Costa, Storia dei lavori dell'Acad. Aspir. Natural., Artic. Zool. (1840); id., Annali dell'Acad. Aspir. Natural. I, p. 4 (1843).

Hermasson minutus, Loew (*nec* Rondani), Stettin. Ent. Zeit. V, p. 115, Pl. I, figs. 1-5 (1844).

Phlebotomus papatasii, Grassi, Mem. d. soc. Ital. d. Sci. (3) XV, p. 353 (1907).

This insect has been described so frequently that it seems unnecessary here to do more than add such particulars as have hitherto been overlooked, or imperfectly dealt with. In the first place it may be noteworthy to state that there are two distinct colour varieties of this common and widely distributed insect:—

(1) A uniformly pale form, which may be considered typical;

(2) A form which differs from the foregoing in having a dark coloured fringe to the costa and hind margin of the wing; herein described as the dark form.

FEMALE.—*Typical pale form* (immediately after death).—Almost uniformly pale translucent ochreous, thorax with a long dull red-brown median stripe, and a single spot of the same colour on either side, near the front margin of the thorax. Hairs on all parts of the body greyish, their arrangement similar to that of the male. Wing relatively broad (fig. 4). Wing fringe not markedly darker than the hairs on the disc of the wing.

MALE.—*Typical pale form* (immediately after death).—Colour similar to that of the female. Clypeus with a tuft of eight to ten hairs; head with a loose tuft, some of the hairs curving forwards, others backwards; tuft on nape of slightly longer ones, chiefly curved forwards. Thorax densely clothed; the hairs arranged in loose tufts. Wing much narrower than in the female (fig. 4). Abdomen uniformly hairy, with small tufts on the dorsum arising from the apical margin of each segment; superior claspers densely hairy, with a few black hairs intermixed with the pale ones; these hairs are easily deciduous, with the exception of a large tuft which is more or less permanent in examples mounted in Canada balsam.

FEMALE.—*Dark form.*—General colour similar to that of the pale form. Wing fringes distinctly smoky grey; some of the hairs on the veins are also dark grey or smoky grey.

MALE.—*Dark form.*—Not observed.

This form is not uncommon; but is very much rarer than the dark form of *P. perniciosus*. It does not differ structurally from typical pale examples so that the following description of the palpi and antennae applies to both varieties.

Palpi of five segments; 1 very short, slightly dilated distally; 2 a little longer than the succeeding one; 3 decidedly broader than the rest; 4 a little shorter than 3; 5 as long as or slightly longer than 2; 1 to 3 hairy; 4 and 5 scaly and with a few fine hairs. *Antennae* (fig. 2) of sixteen segments; 1 and 2 the stoutest, the former with one side longer than the other, the latter bead-like; 3 much the longest, being equal in length to the last five segments together; 4-13 each very slightly shorter than the preceding one respectively; 14 to 16, inclusive, more strongly incrassate (swollen) basally than the rest; all the segments with the exception of 1 and 2 densely clothed with hairs, the longest of which arise from the incrassated portion of each segment, except on the terminal segments which are furnished with hairs of equal length; 4 to 15, inclusive, also furnished with a pair of stout spines (fig. 2), which are suddenly elbowed or bent at right angles to their insertion so that for nine-tenths of their length they lie practically parallel with the surface of the segment to which they are attached.

The external genitalia of the male are much larger than those of any of the other Maltese species; a character which may be readily recognised in life, under a low magnification. The morphological characters are shown in the accompanying illustration (fig. 18). Length, 2·5-2·65 mm.

In captivity this insect is much more restless than *P. perniciosus*, so much so that after a few hours one may readily distinguish the two species by this alone, apart from the other characters; *i.e.*, the generally larger size, paler colour, and much longer legs of *P. papatasii*.

Ovum (Pl. V, figs. 1-5). When forcibly expelled from the body a day or so before the cuticle has become opaque the interior (oolemm) can be seen; and in such examples also the micropyle is distinctly visible as a short ring-like extension at the anterior pole of the egg. The oolemm at this stage is filled with globular particles of fatty matter, suspended in a structureless matrix. When first laid the egg is translucent white and covered with a thin

coating of viscous matter by which it readily adheres to the surface upon which it may fall; five hours after it has been laid it assumes its normal form and colour, which may be described as follows:— Form very elongate, dark brown, shining, with longitudinal black wavy lines, which in certain lights give the periphery of the egg a faintly rugose appearance; these black lines are slightly raised and are joined by slender cross-lines so that a faint but rather coarse reticulation is formed. The transverse lines are, however, very difficult to trace unless they are illuminated by a strong beam of light.

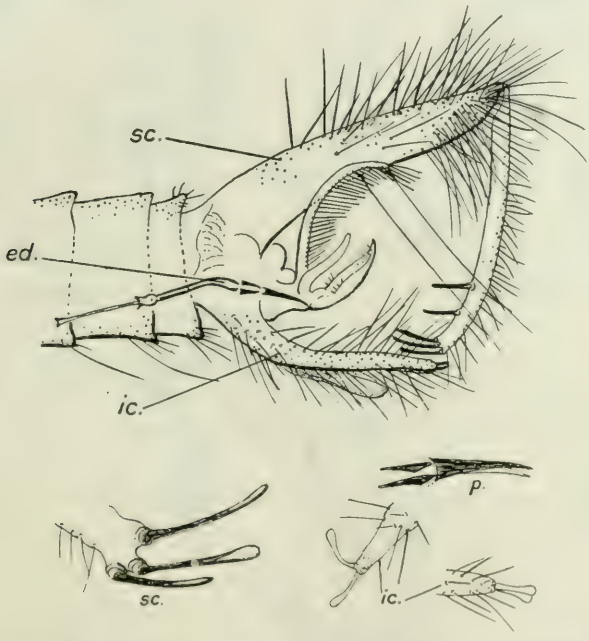


FIG. 18.—External genitalia of *Phlebotomus papatasii*, ♂; *sc*, superior claspers; *ic*, inferior claspers; *ed*, ejaculatory duct; *p*, penis.

The incubation period lasts for about nine days; but unless kept in a moistened atmosphere the eggs will not hatch.

LARVA.—First instar (Pl. V, fig. 8). Cylindrical and distinctly caterpillar-like in its general form; head black; body white or ochreous white; caudal bristles, long, black. Head (fig. 19) very broadly pyriform; frontal hairs two in number; simple; dorsally there are three similar hairs on each side; one arising from the mid-region of the mandibles, one near the base; and a slightly

longer one towards the centre of the head, near the margin; besides these there are at least four hairy spines on each side, arranged as shown in the illustration. Antennae (fig. 19, *ant.*) composed, apparently of three segments, the first two being quite rudimentary and ring-like; third segment broad, flat and ovate in outline, the

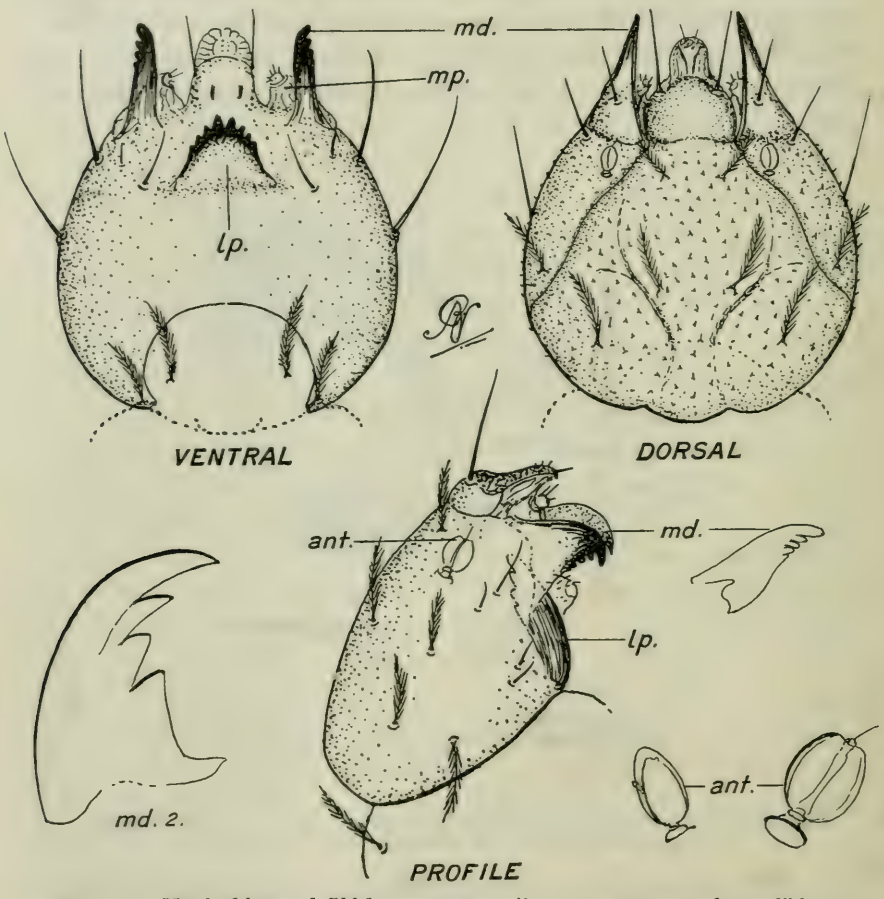


FIG. 19.—Head of larva of *Phlebotomus papatasii*. *ant*, antenna; *md*, mandible; *mp*, maxillary palpus; *lp*, labial plate.

anterior edge faintly emarginate and furnished with a centrally placed hair. Mandibles (fig. 19, *md.*) large and provided with four distinct but rather blunt teeth, of which the apical one is much the largest. Labial plate (fig. 19, *lp*) somewhat triangular in outline with four teeth on each side, the median ones being much the

largest; in its general form the labial plate resembles those found in the larvae of the CULICIDAE. Articulations of the body clearly defined; each segment bears from four to five hairy spines on each side, all of which are broadly dilated apically. Caudal bristles in two pairs, one of which is much the longer, almost equalling the length of the body, the other pair are extremely short.

Last instar (Pl. V, fig. 7). Form resembling that of the first instar; colour pale ochreous white; head black; caudal bristles black, arranged in two pairs, each pair being attached to a large tuberculous process; the inner bristle is much the longer, almost equalling one-half the length of the body of the larva; all of these bristles, under a high magnification, present a number of extremely fine, equidistant, and intensely black surface lines, the intervening spaces being distinctly pale; it is highly probable therefore that these bristles are finely striated, but as no sections were cut it is impossible to determine their true structure by examining them in optical section only. Thoracic and abdominal spines (Pl. V, fig. 10) much longer and stouter than those in the earlier stages; apices *narrowly dilated* and transparent, the remaining portion clothed with minute stiff hairs; these hairy spines are arranged in more or less regular transverse rows, there being four or five on each side of the median line. Head with several large spines similar to those on the abdominal segments, but they are pointed instead of being dilated at the apex; besides these hairy spines there are also several rather long stout hairs, four of which are frontal. Sucker feet similar to, but relatively larger than those in the first instar.

Length 2-3·28 mm.

PUPA.—(Pl. V, figs. 11, 12).—When empty, clear ochreous buff. Eyes in life black. Abdomen curved upwards distally in varying degrees, but not apparently so distinctly **S**-shaped as in *P. perniciosus;* considerably wider in the thoracic region than at the distal segments of the abdomen; integument clothed with minute squamose spines (Pl. V, fig. 15), which are most conspicuous on the abdominal segments. Thorax with two tubercles on each side, the anterior one bearing two or three long slender spines. Abdominal segments each with one (possibly two) extremely minute tubercles at the apex of which is a minute broad flat spine; those on the 7th

and 8th segments more conspicuous than the rest; but all of these processes are so minute as to be easily overlooked. Wing-sheaths pointed apically and extending subventrally as far as the base of the 7th abdominal segment. Head distinctly elongated and somewhat triangular in outline; in the empty pupa this often breaks away in the process of mounting when the outline may be seen to bear a striking resemblance to the head of an ox in miniature (Pl. V, fig. 13). Antennal sheaths distinctly segmented, lying curved behind the eyes and subsequently following the costa of the wing-sheath. Palpal sheaths originating near the centre of the frons, extending backwards and then curving suddenly forward so that the apex rests against the antennal sheath and lies pointing in the same direction. Legs extending slightly beyond the wing sheaths.

ACKNOWLEDGMENTS

The Liverpool School of Tropical Medicine is much indebted to the Tropical Diseases Research Fund (Colonial Office) for the Grant of £100 towards defraying the cost of the expedition, and to the representatives of the Moss Steam Ship Company for granting a free passage by their boats. I wish also to express my thanks for assistance rendered in the furtherance of the objects of the Expedition by His Excellency the Governor and Commander-in-Chief, General Sir Leslie Rundle, K.C.B., K.C.M.G., D.S.O., etc., who very kindly gave permission to visit all Government lands and Institutions and also sanctioned the use of the Laboratory at the Public Health Department; to the Honourable E. C. Roupell, D.S.O., Acting Lieutenant Governor, for much valued assistance and kind hospitality; to the officials of the Civil, Naval and Military Departments; to the Hon. C. Caruana Scicluna, Chief Government Medical Officer, and the members of his staff, especially to Professor T. Zammitt, who furthered the work of the expedition in every possible way; to the officers of the Royal Army Medical Corps, Captain Babbington, Captain Lloyd-Jones, Captain Steward, Captain Beaman, and especially to Captain Marett, R.A.M.C., for placing the whole of his valuable material in my hands for examination; to Major G. S. Crawford, R.A.M.C., I am

also specially indebted for valuable aid and for his kind hospitality; to Major F. L. Dibblee, Royal Marine Artillery and Mrs. Dibblee for collecting extensive series of material for investigation; to Surgeon Lancelot Kilroy, H.M.S. Diana, for examples of Simulium from Crete; to the Government Veterinary Officer, Dr. A. M. Macfarlane, for a valuable collection of Helminths and other intestinal parasites for demonstrative and other purposes in the Laboratory and Museum of the Liverpool School of Tropical Medicine.

REFERENCES

1. Annandale, N. (1910). Records of the Indian Museum, Vol. V, Pt. 3, Nos. 13 and 14.

2. Birt, C. (1910), 'Phlebotomus Fever in Malta and Crete.' Journal of the Royal Army Medical Corps, Vol. XIV, pp. 236-258.

3. Grassi, B. (1907), 'Ricerche sui Flebotomi.' Memorie della Società italiana delle Scienze, Ser. 3a, XIV, pp. 353-395.

4. Grassi, B. (1908), 'Intorno ad un nuovo Phlebotomo.' Rend. Reale Accademia dei Lincei Ser. 5a, XVII, pp. 618-682.

5. Howlett, F. M. (1909), 'Indian Sandflies.' Indian Medical Congress, Section III, pp. 239-242.

6. Marett, P. J. (1910), 'Preliminary report on the investigation into the breeding places of the sand-fly in Malta.' Journal of the Royal Army Medical Corps, XV, 3, pp. 286-291.

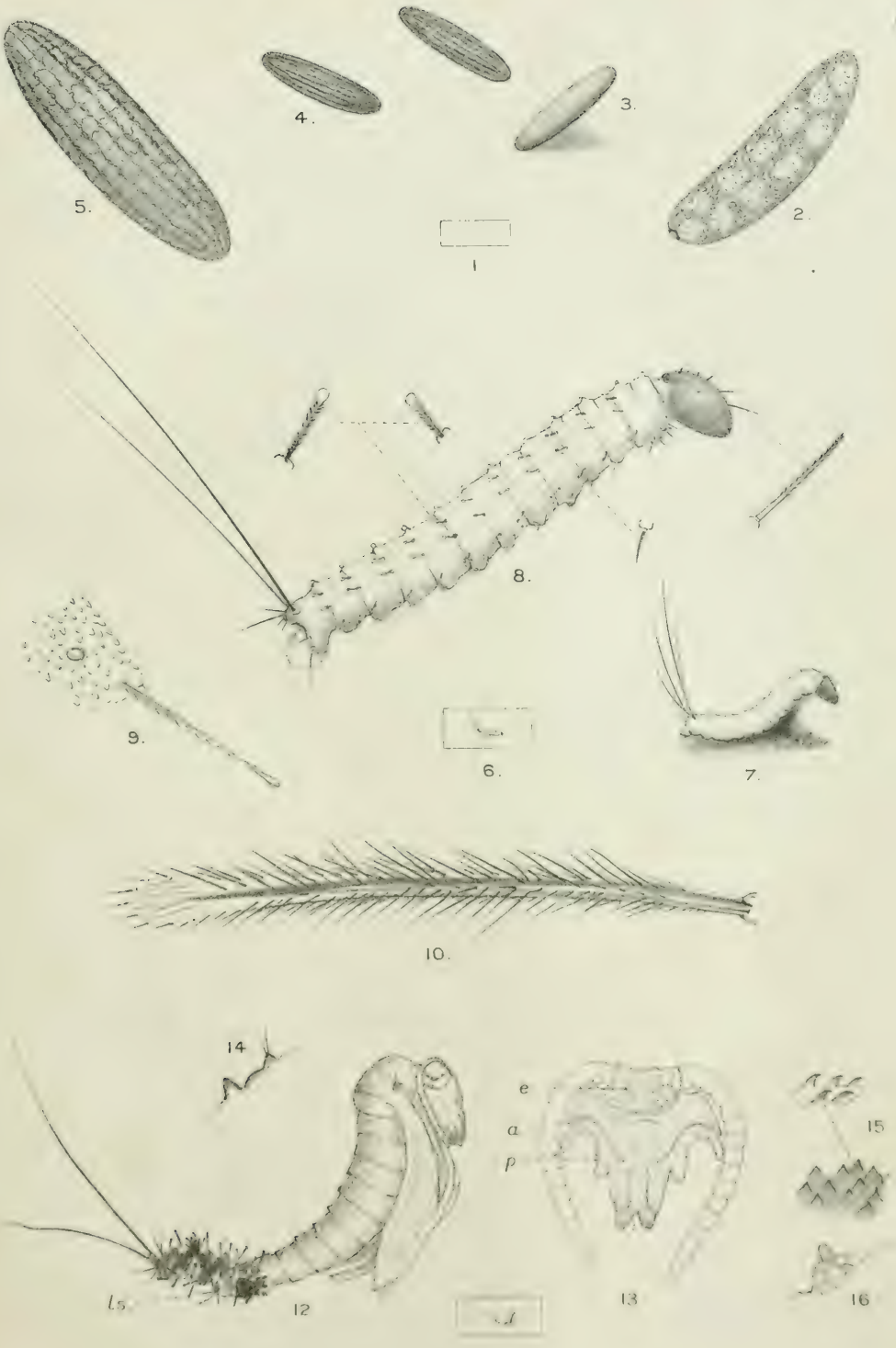
7. Neveu-Lemaire, M. (1906), Bulletin de la Societé de France, p. 65.

8. Speiser, P. (1910), Zoologische Jahrbücher, Supp. 12, Heft. 3, Taf. 22.

PLATE V

Phlebotomus papatasii, Scop.

Fig. 1. Eggs, approximately natural size.
2. Egg, a few hours before extrusion, showing micropyle.
3. Egg, freshly extruded.
4. Egg, a few hours after extrusion.
5. Egg, much enlarged, to show reticulated surface.
6. Larva, approximately natural size.
7. Sketch of adult larva, enlarged.
8. Larva; first instar, enlarged.
9. Stigma of larva with spine.
10. Hairy spine of larva.
11. Pupa, approximately natural size.
12. Pupa enlarged: *ls*, larval skin with anal bristles attached.
13. Front view of the head of the pupa: *e*, eye; *a*, antenna; *p*, palpus.
14. Thoracic tubercles of pupa.
15. Squamose body-spines of pupa.
16. One of the abdominal papillae of the pupa.



R. Newstead, *ad. nat. del.*

Bale & Danielsson, Ltd., *lith.*

PHLEBOTOMUS PAPATASII

PLATE VI

Phlebotomus papatasii, Scop.

Fig. 1. Imagos, approximately natural size.
2. Male, enlarged; from life.

Phlebotomus perniciosus, Newst.

3. Pupa, approximately natural size.

4. Pupa, enlarged.

5. One of the abdominal tubercles of the pupa.

6. Squamose spines of the abdominal segments of the pupa.







R. Newstead, *ad. nat. del.*

Bale & Danielsson, Ltd., *lith.*

PHLEBOTOMUS PAPATASII AND P. PERNICIOSUS.

PLATE VII

Fig. 1. *Phlebotomus papatasii*, Scop., female, enlarged; from life.

2. *Phlebotomus perniciosus*, Newst., female, enlarged; from life.

3. *Phlebotomus perniciosus*, approximately natural size.

[Note.—The above enlarged figures and that of the male shown on Plate VI are all drawn to the same scale.]



1.



2

3

R. Newstead, *ad. nat. del.* Bale & Danielsson, Ltd., *lith.*

PHLEBOTOMUS PAPATASII AND P. PERNICIOSUS.

THE EXPERIMENTAL TRANSMISSION OF GOITRE FROM MAN TO ANIMALS

BY

ROBERT McCARRISON, M.D., M.R.C.P. (Lond.),

CAPTAIN, INDIAN MEDICAL SERVICE,
AGENCY SURGEON, GILGIT, KASHMERE

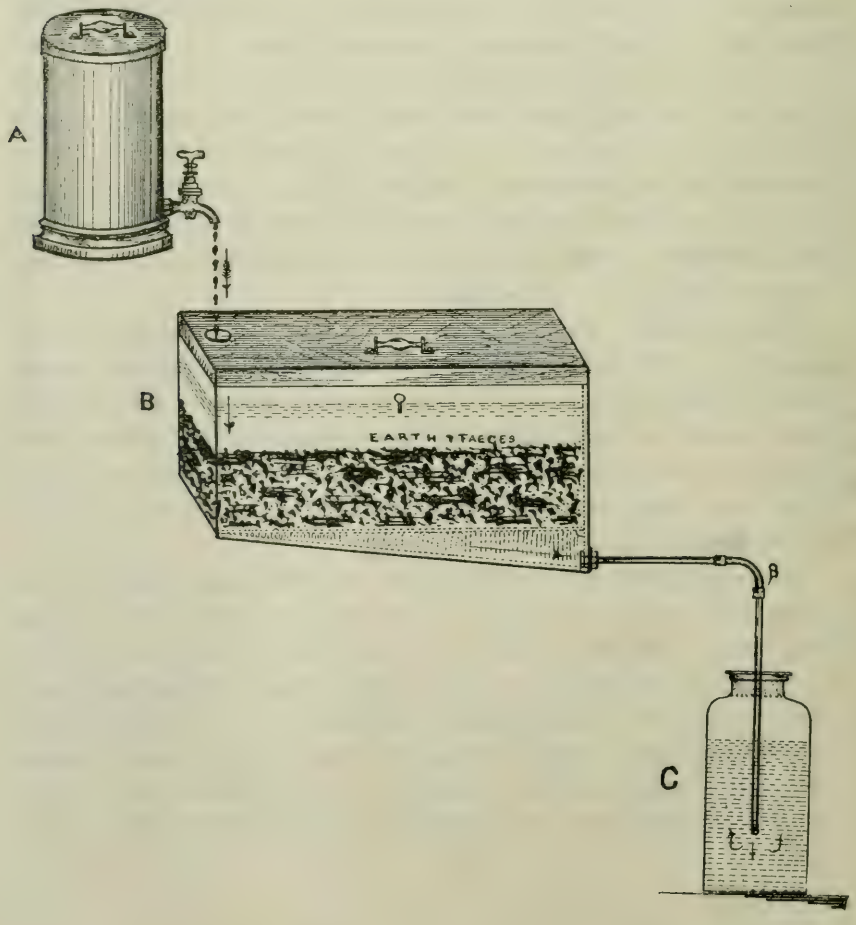
(*Received for publication* 20 *April*, 1911)

Since the year 1906 I have repeatedly endeavoured to transmit goitre from man to animals by infecting the water supply of the latter with the faeces of sufferers from goitre. The assumption that goitre can be so conveyed follows upon the results of my former researches. I had previously employed dogs, but obtained no results of a positive character (1906-11). This was due largely to the fact that dogs suffer little from goitre in this country, though these animals are known to be frequently affected in other localities. I believe that the comparative freedom of dogs from goitre in Gilgit indicates that the infecting agent of this disease is less toxic there than elsewhere. Other facts point in the same direction, but these I hope to discuss in another place.

The possibility that an intermediate host was concerned in the spread of goitre having occurred to my mind, I suspected earthworms of playing this rôle. I, consequently, devised an experiment which would not only again test the assumption that goitre could be transmitted from man to animals by infected faeces, but would at the same time determine the influence, if any, which earthworms might possess in spreading the disease.

Female goats were selected for this dual experiment. The goats were between the ages of one and two years and were not pregnant. This latter point is of importance, as pregnancy, owing to the increased activity of the thyroid gland during that state, would vitiate the results observed on microscopical examination of this organ. The goats were brought from a non-goitrous locality, high up in the mountains, about forty miles distant from Gilgit. Their thyroid glands were carefully examined and were found to

show no signs of hypertrophy. In the majority the gland on each side could be felt only with the greatest difficulty, or could not be distinguished from the surrounding tissues. Where the organ was palpable it was found to be oval in shape and about the size of a very small almond. The goats were divided into batches, which will be referred to as: I, 'Controls' (three goats); II, 'Batch X' (six goats); III, 'Batch Y' (seven goats). Each batch was confined in a separate house, the door of which was of netted wire. The animals were all fed on the leaves and young branches of trees, in order to exclude as far as possible sources of contamination from the soil. The experiment was designed so as to foul the drinking water of 'Batches X and Y,' while that of the 'Controls' remained pure. The apparatus which I employed for this purpose is represented in the figure:—



It consisted of a covered drum, A, which was fitted with a tap; a covered wooden box, B, having a perforated false bottom of wire gauze under which was a wooden trough sloping towards an outlet pipe β. This outlet pipe led into a flask, C, which was fitted with a perforated cork so as to ensure freedom from contamination by soil, dust, etc. The drum A was filled with a non-goitre producing water which was previously boiled. A trickle of water from the drum A was allowed to pass continuously into the box B. This box was partially filled with soil taken from the most goitrous village in Gilgit. The soil was sterilized by steam at 230° F. for thirty minutes, and was then mixed with the faeces of sufferers from incipient goitre. Fresh faeces were added to the mixture several times a week during the course of the experiment. The water from the drum A trickled into the box B, saturated the mixture of sterilized earth and faeces, and, passing through the perforated bottom of the box, found its way, by means of the wooden trough and outlet pipe β, into the corked flask C. This was the only drinking water provided for the goats of 'Batches X and Y.'

A separate apparatus was used for each batch. In the case of 'Batch Y,' however, 500 earthworms were introduced into the box B. It will be seen that 'Batch X' were provided with drinking water which was grossly contaminated with the faeces of sufferers from goitre, while in the case of 'Batch Y' the drinking water contained also such additional matter as was derived from the excreta of the earthworms. The water which found its way into the stoppered bottle was in both cases foul smelling and of a dark grey colour.

The experiment was commenced on the 13th October, 1910, and terminated on the 15th December, 1910. The thyroid glands of the control goats showed no change during the sixty-four days of the experiment. In the case of the goats of 'Batches X and Y,' however, it was observed that

(1) the animals lost in weight, due doubtless to confinement in a small hut for sixty-four days;

(2) many of them suffered from diarrhoea;

(3) 50 per cent. of the goats in each batch showed enlargements of the thyroid gland, which was most marked in the right side.

To facilitate examination of the necks of these animals the hair was kept closely clipped, but as measurement and photography cannot be employed in the case of animals as aids to diagnosis, palpation alone had to be relied upon during the course of the experiment. It was observed that the thyroid gland in two goats in 'Batch X' showed signs of enlargement as early as the thirteenth day of the experiment. In all cases in which enlargement occurred, a noticeable feature was the manner in which the size of the gland fluctuated. At one examination it would be found that the organ was little, if anything, larger than in the case of the controls, while a day or two later it would appear to be not less than twice the size. Goitre in man, whether artificially produced or naturally acquired, shows the same tendency to fluctuate in size in its early stages (1909) (1911, Febr.). The animals were killed on the 15th December by a Goorkha, skilled in severing the head from the body at one stroke of his kookrie (knife). The neck was dissected immediately and the gland rapidly exposed. It was observed in several cases that the size of the organ diminished considerably before it could be removed from the body. The right and left lobes of the thyroid, with their long and narrow isthmus, were rapidly dissected out and weighed. The following table shows the results in the case of the sixteen animals employed in the experiment:—

I.—'Controls,' 3 in number, which consumed only pure water:—

Weight of goat	Weight of thyroid	Proportionate weight of thyroid to body weight
1 ... 60 lbs.	3·2 gms.	$\frac{1}{10,000}$
2 ... 43 ,,	2·05 ,,	$\frac{1}{10,439}$
3 ... 65 ,,	3·2 ,,	$\frac{1}{10,100}$

II.—'Batch X,' 6 in number, which consumed only faecally contaminated water :—

	Weight of goat	Weight of thyroid	Proportionate weight of thyroid to body weight
1	... 48½ lbs.	3·6 gms.	$\frac{1}{6,654}$
2	... 26½ ,,	2·93 ,,	$\frac{1}{4,500}$
3	... 38 ,,	2·3 ,,	$\frac{1}{8,000}$
4	... 30 ,,	1·5 ,,	$\frac{1}{10,000}$
5	... 22½ ,,	2·32 ,,	$\frac{1}{4,800}$
6	... 29 ,,	1·35 ,,	$\frac{1}{10,700}$

III.—'Batch Y,' 7 in number, which consumed faecally contaminated water, as in the case of 'Batch X,' together with the excrementitious products of earthworms :—

	Weight of goat	Weight of thyroid	Proportionate weight of thyroid to body weight
1	... 50½ lbs.	4 gms.	$\frac{1}{7,000}$
2	... 41½ ,,	4·3 ,,	$\frac{1}{4,850}$
3	... 56 ,,	3·7 ,,	$\frac{1}{7,530}$
4	... 55½ ,,	3·6 ,,	$\frac{1}{7,700}$
5	... 49½ ,,	5·4 ,,	$\frac{1}{4,272}$
6	... 45 ,,	1·9 ,,	$\frac{1}{11,700}$
7	... 39 ,,	1·9 ,,	$\frac{1}{10,000}$

It will be seen from the tables that the weight of the control animals' thyroid was in all cases about $\frac{1}{10,000}$th part of the body weight. In 'Batch X' the thyroids of Nos. 4 and 6 showed no deviation from the normal weight. In two, Nos. 1 and 3, the

weight of the thyroid was considerably more than normal, while in the remaining two, Nos. 2 and 5, it was twice as much as that of the controls. In 'Batch Y' Nos. 6 and 7 had thyroid glands of the normal weight; in Nos. 1, 3 and 4 the weight of these organs was considerably more than normal; while in Nos. 2 and 5 the thyroids were more than twice the weight of those of the control animals. Allowing for variations in size of the normal thyroid of from $\frac{1}{8,000}$th to $\frac{1}{11,000}$th part of the body weight, it will be admitted that about 50 per cent. of the animals in 'Batches X and Y' showed enlargements of the thyroid gland.

MICROSCOPICAL APPEARANCES OF THE GLANDS

Striking differences were observed in the microscopical appearances of the thyroids in these animals. In the control animals the vesicles were found to be small, round, compact, and lined with cubical epithelium. The space between the vesicles was filled with masses of round cells, and many vesicles were seen in the field of the microscope. In the thyroids of those animals which showed the greatest increase in size as determined by weight, the vesicles were much larger, their walls markedly thinner, and their outline much more irregular, while fewer appeared in the field of the microscope than in the case of the normal gland. The epithelium lining the vesicles was much flattened, and the intervesicular cellular tissue was markedly less in amount than in the case of the normal gland. Sections of the enlarged organs often showed fields in which the colloid had fallen out during the process of staining; these fields, when viewed as a whole, had a peculiar 'netted-wire' appearance, which differed very markedly from the compact structure of the normal gland. Glands which showed an intermediate degree of enlargement presented in some parts of the section the appearances of normal tissue, while in others the dilated vesicles, flattened epithelial lining and the scarcity of intervesicular cellular tissue were characteristic of the more enlarged organs. In short, every degree of variation was met with from the small, round, compact vesicle of the normal organ to the cyst-like dilatation of the vesicle of the hypertrophied gland. In none of the enlarged organs was there any evidence of active cell proliferation or of inflammatory change. There appeared to be little or no alteration in the connective tissue stroma of the enlarged glands.

The increase in size of the hypertrophied organs was due wholly to the distension of the vesicles with colloid material. It seems evident that this distension must result in thinning and rupture of the walls of the vesicles and in the formation of small, cystic cavities in the gland; and that distension of existing vesicles, and the formation of new vesicles from the intervesicular cell masses, are the earlier stages in the development of parenchymatous goitre. In some cases colloid was seen in the vessels of the gland (Photomicrograph VI).

This experiment proves that a hypertrophy of the thyroid gland of goats can be induced by infecting the water supply with the faeces of sufferers from goitre. It is, at present, impossible to state whether this hypertrophy is due to the action of the infecting agent of goitre, or only to the organic impurity of the water thus contaminated.

Earthworms do not appear to be concerned in the spread of goitre.

Goitre is essentially a disease of country localities, that is, of localities of unprotected water supplies. In this country I have found, from a study of the topography of a very large number of villages, that the freedom, or the reverse, of any particular village from this disease depends very largely on the extent to which the drinking water is contaminated by irrigating fields, which are fertilized with human and animal excreta. The source of the water supply and the geological formations from which it is derived are, in comparison, of minor importance.

Further experiments on goats are at present in progress, with a view to confirming the results detailed above. These experiments have not long been in progress, but already five out of twelve goats which are drinking faecally contaminated water show signs of enlargement of the thyroid gland, while no changes have occurred in the thyroids of twelve control animals. The final results of these experiments and the microscopical appearances of the glands will be detailed in a subsequent report.

REFERENCES

McCarrison, R., (1906), Lancet, Dec. 8.
————,—, (1908), Proc. Roy. Soc., B. LXXXI.
————,—, (1909), Quart. Journ. Med., II, 7.
————,—, (1911), Proc. Roy. Soc., Febr. 28, B. LXXXIII.
————,—, (1911), Ann. Trop. Med. Parasit., Apr. 20, V, 1.

DESCRIPTION OF PLATES

Plate VIII

Fig. 1.—Photomicrograph of normal thyroid gland of goat. × 100. Shows numerous rounded vesicles holding colloid. The cubical epithelial lining and the cell masses between the vesicles cannot be seen with this magnification. Section taken from the thyroid gland of control goat No. 2.

Fig. 2.—Photomicrograph of artificially produced parenchymatous goitre in goat. × 100. Shows the marked dilatation of the vesicles and the thinning of their walls. Section taken from thyroid gland of goat No. 1, 'Batch Y.'

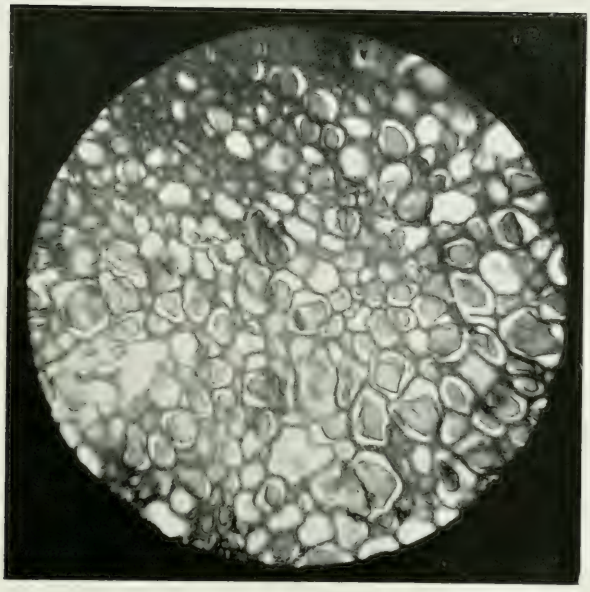


FIG. I

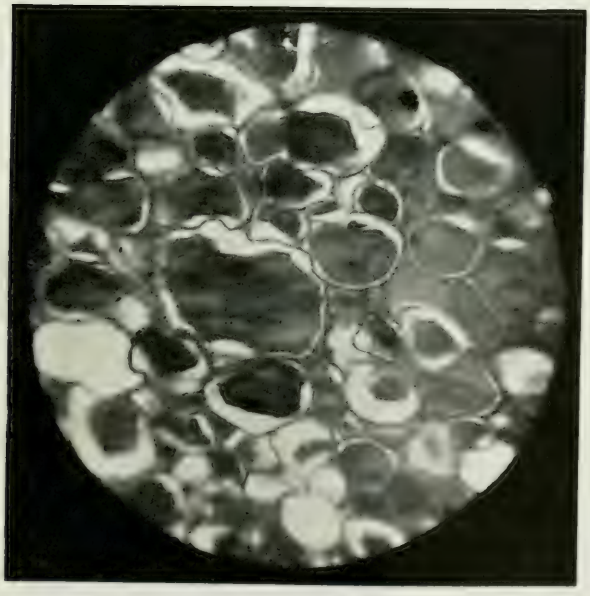


FIG. II

Plate IX

Fig. 3.—Photomicrograph of normal thyroid gland of goat. × 100. Shows same appearances as in Photomicrograph No. 1. Section taken from thyroid gland of goat No. 6, 'Batch X.'

Fig. 4.—Photomicrograph of artificially produced parenchymatous goitre in goat. × 100. Shows marked dilatation of the vesicles, thinning of the vesicle walls, and the 'netted-wire' appearance alluded to in the text. Section taken from thyroid gland of goat No. 5, 'Batch Y.'

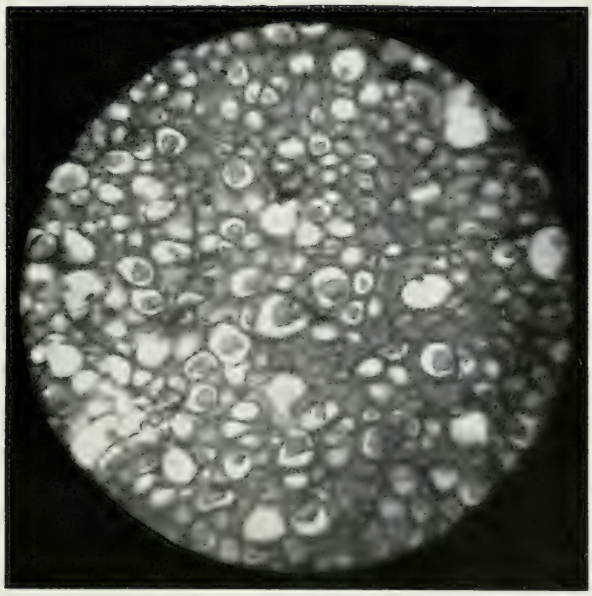


FIG. III

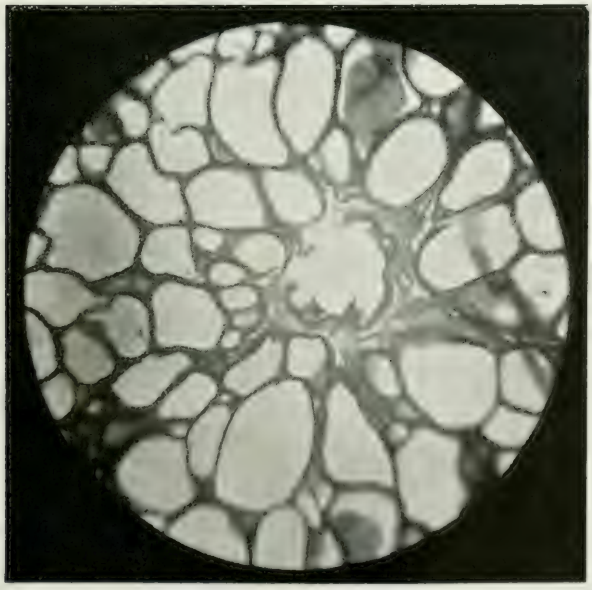


FIG. IV

Plate X

Fig. 5.—Photomicrograph of normal thyroid gland of goat. × 100. The cubical epithelial lining can be seen in a few of the vesicles. Figs. 1, 3 and 5 illustrate the variations in the size of the vesicles which are met with in the normal thyroid gland of the goat. Section taken from thyroid gland of control goat No. 5.

Fig. 6.—Photomicrograph of artificially produced parenchymatous goitre in goat. × 100. Shows colloid in vessel of gland. Part of the field shows comparatively normal appearances. Photograph was taken from the same section as Fig 2.

The sections, from which the photomicrographs were taken, were stained in aqueous solutions of magenta red, picric acid and indigo carmine (Borrel's stain).

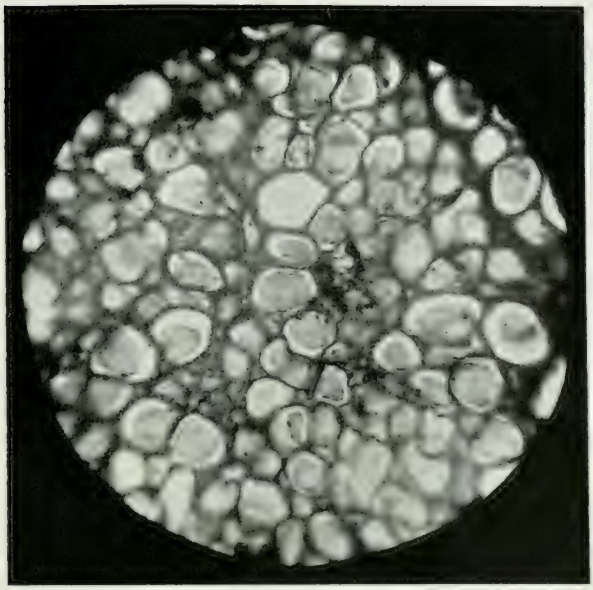


FIG. V

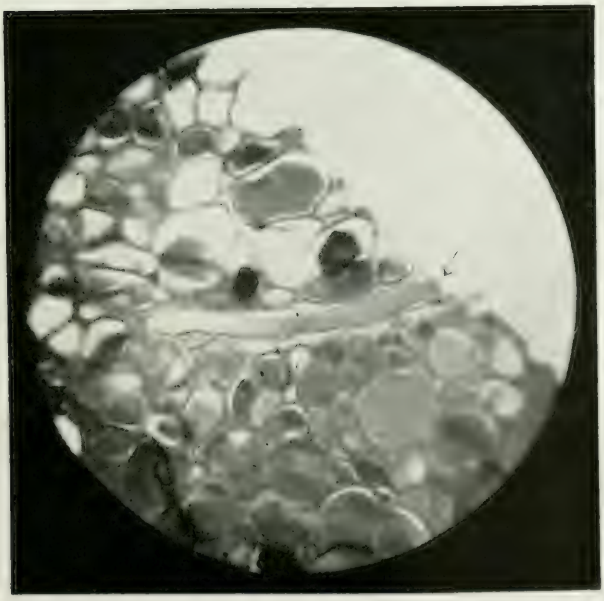


FIG. VI

REDUCING ACTION OF TRYPANOSOMES ON HAEMOGLOBIN

BY

RALPH W. NAUSS

AND

WARRINGTON YORKE

From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine.

(*Received for publication* 21 *April*, 1911)

Amongst the blood changes occurring in animals infected with trypanosomes there is one which, so far as we can ascertain, has escaped observation. Whilst performing experiments with the object of investigating the cause of auto-agglutination of red blood cells*—a phenomenon which is frequently to be observed in cover-slip preparations of the fresh blood of infected animals—it was observed by one of us (W.Y.) that the colour of the erythrocytes in certain of the capillary tubes had changed from the normal red tint to a deep purple.

Further examination showed that this alteration in colour only occurred when the plasma, which had been added to the suspension of washed erythrocytes in normal saline solution, contained a considerable number of actively motile trypanosomes. The appearance was most striking in the tests carried out at 37° C. It was not so distinct in the tubes kept at 15° C., and was absent from those which had been placed in the ice-chest.

The fresh blood of a large number of animals of different species in various stages of infection was examined as regards its colour.

Technique.—A few drops of blood were allowed to fall from a vein of the ear into a tube containing a small amount of citrated saline solution. The red blood cells were then quickly thrown down by centrifugalisation and their colour noted. As a rule the

* Yorke. 'Auto-agglutination of Red Blood Cells in Trypanosomiasis,' Roy Soc. Proc., 1910, Vol. LXXXIII, p. 238.

purple colour, when present, could easily be seen as the blood escaped from the wounded vein.

Only comparatively rarely were the red blood cells found to exhibit a markedly purple colour. It was occasionally seen in infected rats when the parasites in the peripheral blood were exceedingly numerous. As a rule, however, the blood of these animals was found to present the normal red colour even when it was swarming with trypanosomes.

Durham,* in a recent paper, has drawn attention to this fact. He states that, in rats suffering from Nagana, the blood in an advanced stage of the disease is sometimes of a dull purplish or chocolate colour. Examination with the spectroscope showed the bands of oxyhaemoglobin.

The blood of some of our infected rabbits exhibited this change of colour in a marked degree during the later stages of the disease. In the case of a rabbit infected with *T. rhodesiense*, the blood, as it escaped from the incised vein, was of an exceedingly purple colour.

The blood of this animal was subjected to various tests with a view to obtaining information as to the cause of this appearance.

In the first place it was found that when the blood was thoroughly shaken with air, or, when air was blown through it, the dull purple tint gradually gave place to the bright red colour of normal oxyhaemoglobin, until, finally, the appearance was identical with that of normal blood. The conversion of the purple colour into the red was not particularly rapid, and the blood required to be intimately mixed with air before it was accomplished. The fact that the red blood cells could be washed many times in large volumes of normal salt solution and still retain a distinctly purple colour illustrates how stable is the condition.

It will be noted that this observation is at variance with the results obtained by Durham with the blood of rats infected with *T. brucei*. Durham states that the dull coloured blood of these animals may be shaken with air, or allowed to stand for a week or more without developing the full red of normal oxyhaemoglobin.

Spectroscopic examination of a solution made by adding a few

* 'Notes on Nagana and some Haematozoa observed during my travels,' Parasitology, 1908, p. 227.

drops of the blood of this rabbit to distilled water revealed the ordinary bands of oxyhaemoglobin. No abnormal absorption bands were seen. When, however, the blood was added to previously boiled distilled water, avoiding as far as possible contact with air, the spectroscopic appearances were very different. Well-marked bands of oxyhaemoglobin were visible as before, but the space between the bands was also considerably darkened and the zone of absorption extended further towards the red. In other words, it was obvious that instead of the spectrum of a pure solution of oxyhaemoglobin we were dealing with that of a solution of partially reduced haemoglobin.

The dull purple colour of the blood is due, therefore, to the presence of a certain proportion of haemoglobin in the reduced form.

Some years ago Ehrlich demonstrated the ability of living cells to decolourise solutions of methylene blue.

Later it was shown by Klett,* Neisser and Wechsberg† and others that living leucocytes, spermatozoa, pancreas and kidney cells, and various micro-organisms all possessed this property. Neisser and Wechsberg found that these cells and bacteria lost their reducing capacity after they had been treated with toxic substances.

Ricketts‡ observed that emulsion of nervous tissue caused reduction of a solution of methylene blue. Further investigation indicated that intact cells were not essential for this reduction, and that emulsions which had been kept in the ice-chest for a week still reduced, although less vigorously than when fresh.

Experiments were performed by us with the object of ascertaining whether actively motile trypanosomes had a reducing action on solutions of methylene blue.

Solutions of various concentration were prepared by dissolving the dye in water to which sufficient sodium chloride had been added to make it isotonic. Small cells of a capacity of approximately 1 c.c. and 10 mm. in depth were completely filled with a mixture

* 'Zur Kenntniss der reducirenden Eigenschaften der Bakterien,' Zeit. für Hygiene, 1900, S.137.

† Ueber eine neue einfache Methode zur Beobachtung von schädigungen lebender Zellen und Organismen (Bioskopie),' Münch. med. Woch., 1900, S.126.

‡ 'Reduction of Methylene Blue by Nervous Tissue,' Journal of Infectious Diseases, 1904, Vol. I, p. 590.

consisting of equal portions of citrated plasma containing many actively motile trypanosomes in suspension and the isotonic solution of methylene blue. The cells were then sealed with cover-slips and placed in the incubator at 37° C.

It was found that in the course of a few minutes the fluid in the cells containing numerous trypanosomes and only weak solutions of methylene blue (0·05 per cent., or less) had completely lost its bluish tint and become colourless, indicating that complete reduction of the dye had resulted. On the other hand, no reduction was observed in the cells which contained the more concentrated solutions of methylene blue. It was found that solutions of the dye equal to 0·5 per cent. speedily caused the parasites to become motionless and die.

In view of the injurious effect of solutions of methylene blue on the parasites we were obliged to seek another indicator, the presence of which did not prevent the trypanosomes from carrying out their physiological functions.

Ultimately, it was decided to use isotonic solutions of rabbit haemoglobin. Solutions of this substance were found to possess many advantages.

(1) It had no injurious action on the parasites.

(2) The strength of the solution could be easily measured, and we were thus enabled to perform experiments of a quantitative character.

(3) An obvious change of colour occurs when such solutions are undergoing reduction.

(4) By the aid of the spectroscope it is possible to determine when reduction is complete.

Technique.—Blood from a vein of the ear of a rabbit was allowed to drop into citrated saline solution. The red blood cells were then separated from the citrated plasma by centrifugalisation, and subsequently washed several times with physiological salt solution. The washed erythrocytes were then laked by the addition of distilled water, and after the lapse of a few minutes sufficient sodium chloride was added to render the solution isotonic. A light precipitate, consisting for the most part of red cell stromata, appeared upon the addition of the salt and was thrown

down by means of the centrifuge and the clear solution of haemoglobin withdrawn.

The haemoglobin content of the solution in terms of human wet red cells was then determined by means of a haemoglobinometer reading.*

The suspension of trypanosomes was obtained by adding four volumes of the blood of an infected animal to one volume of a solution containing 1 per cent. sodium citrate and 0·9 per cent. sodium chloride. The red corpuscles having been thrown down by the aid of the centrifuge, the citrated plasma containing the trypanosomes in suspension was decanted off. The number of parasites present was determined by suitably diluting a small portion with sodium chloride solution and counting by means of a Thoma Zeiss haemocytometer.

A spectroscope tube of known capacity and 10 mm. in height was then completely filled with a mixture consisting of equal parts of the haemoglobin solution of known strength and of the suspension of trypanosomes containing a definite number of parasites per cubic millimetre. The tube was then sealed with a cover-slip and placed in the incubator at 37° C.

Control experiments were always made with the same solution of haemoglobin and the plasma of a normal animal of the same species diluted to a similar degree as the infected plasma.

After the expiration of only a few minutes a distinct change in colour was observed in the tubes which contained the suspension of trypanosomes. The bright red of the oxyhaemoglobin was being replaced by the dull purple colour of reduced haemoglobin. This process continued, until, finally, the colour became dark purple.

From time to time the tubes were examined spectroscopically by means of a Zeiss comparison spectroscope and the absorption bands compared with those of the standard solution of oxyhaemoglobin.

Since the contents of all the tubes gave absorption bands practically identical in appearance at the beginning of the experiment it was easily determined in which of the tubes changes

* Barratt and Yorke. 'An Investigation into the Mechanism of Production of Blackwater,' Annals of Tropical Medicine and Parasitology, 1909, Vol. III, p. 14.

were occurring by comparison with the standard solution which was kept in contact with the air at 15° C.

As the colour of the solutions became more and more purple the oxyhaemoglobin bands became less well defined. The space between the bands at D and E became darker, whilst the zone of absorption embracing the D line appeared to extend further towards the red. Finally, in place of the two distinct bands of oxyhaemoglobin there was only the single band of reduced hæmoglobin.

As will be seen from Table 1 the rapidity and degree of reduction varied, as a rule, directly with the number of parasites present in the citrated plasma. There were, however, certain well-marked exceptions. In Experiment 12, where the citrated plasma was derived from a rat heavily infected with *T. equiperdum*, there was scarcely any reduction of the haemoglobin solution, in spite of the fact that the infected plasma solution contained numerous trypanosomes (300,000 per c.mm.). When cover-slip preparations of the blood of this rat were examined it was found that the parasites were sluggishly motile and quickly clumped together into large masses and became motionless. Other analogous observations indicate that for appreciable reduction to occur it is essential for the trypanosomes to be actively motile. Trypanosomes killed by heating for a short time to 50° C. caused no reduction of methylene blue or haemoglobin solutions.

Having determined that actively motile trypanosomes exert a marked reducing action upon haemoglobin, we decided to continue our investigations with a view to ascertaining so far as possible in what manner the gaseous contents of the blood are altered by the action of living trypanosomes.

With this object in view, an analysis was made of the gases contained in a definite volume of defibrinated rabbit blood which had been treated for one hour at 37° C. with a certain amount of citrated plasma, containing numerous living trypanosomes. In the control experiments a similar volume of the same defibrinated blood was treated for a like time with an equal quantity of citrated plasma from a normal animal of the same species as the animal yielding the infected plasma. As a further control the gases present in the defibrinated blood alone were determined.

Table 1. Reducing action of trypanosomes on haemoglobin.

No. of experiment	Variety of trypanosomes	Equal Portions of Infected Plasma and Haemoglobin Solution		Time in which complete reduction occurred
		Number of parasites per cubic millimetre	Strength of haemoglobin solution	
1	*T. gambiense* ...	10,000	0·73 %	Complete in 1 hour
2	,, ...	18,250	0·75 %	Partial in 15 minutes
3	,, ...	30,000	0·86 %	Complete in 1 hour
4	,, ...	36,500	0·75 %	Complete in 10 minutes
5	,, ...	600,000	0·72 %	,,
6	*T. brucei*	300	0·75 %	Nil in 1 hour
7	,, ...	32,000	1·4 %	,,
8	,, ...	40,000	0·75 %	Complete in 10 minutes
9	*T. evansi*	3,000	1·0 %	Nil in 1 hour
10	,, ...	12,000	0·74 %	Complete in 15 minutes
11	*T. equiperdum* ...	100,000	0·72 %	,,
12	,, ...	300,000	1·00 %	Nil in 1 hour
13	*T. equinum*	100,000	3·4 %	Complete in 20 minutes
14	,, ...	170,000	3·4 %	Complete in 15 minutes

N.B.—In the control experiments where normal plasma was used instead of that of infected animals no reduction occurred.

TABLE 2. Analysis of gases obtained from defibrinated blood and from mixtures of this with plasma of normal and infected animals after incubation at 37° C. for 1 hour in the absence of air.

No. of experiment		Composition of Blood Mixture		Analysis of Gases obtained from Blood Mixture after Heating for 1 hour at 37° C. in the Absence of Air					
		Amount of defibrinated blood of normal rabbit	Amount of citrated plasma of normal or infected animal consisting of 2 parts plasma and 1 part citrated saline solution	Total	CO_2	O	N	Deficiency in amount of oxygen in C as compared with B	Variation in amount of carbon dioxide in C as compared with B
1	A	3 c.c.	—	0·730 c.c.	0·40 c.c.	0·28 c.c.	0·05 c.c.	—	—
	B	,,	1·5 c.c. from normal rat	1·00 c.c.	0·60 c.c.	0·275 c.c.	0·125 c.c.	—	—
	C	,,	1·5 c.c. from rat infected with *T. equiperdum* containing 640,000 trypanosomes per c.mm.	0·745 c.c.	0·645 c.c.	0·025 c.c.	0·075 c.c.	0·25 c.c.	0·045 c.c.
2	A	3 c.c.	—	0·775 c.c.	0·390 c.c.	0·33 c.c.	0·055 c.c.	—	—
	B	,,	1·5 c.c. from normal guinea-pig	0·97 c.c.	0·57 c.c.	0·29 c.c.	0·11 c.c.	—	—
	C	,,	1·5 c.c. from guinea-pig infected with *T. brucei*, containing 340,000 trypanosomes per c.mm.	0·76 c.c.	0·67 c.c.	0·007 c.c.	0·08 c.c.	0·283 c.c.	0·1 c.c.
3	A	3 c.c.	—	0·64 c.c.	0·34 c.c.	0·26 c.c.	0·04 c.c.	—	—
	B	,,	1·5 c.c. from normal guinea-pig	1·14 c.c.	0·76 c.c.	0·315 c.c.	0·075 c.c.	—	—
	C	,,	1·5 c.c. from guinea-pig infected with *T. brucei* containing 450,000 trypanosomes per c.mm.	0·65 c.c.	0·58 c.c.	0·00 c.c.	0·07 c.c.	0·315 c.c.	−0·18 c.c.
4	A	3 c.c.	—	0·67 c.c.	0·305 c.c.	0·31 c.c.	0·055 c.c.	—	—
	B	,,	1·5 c.c. from normal guinea-pig	0·91 c.c.	0·535 c.c.	0·305 c.c.	0·07 c.c.	—	—
	C	,,	1·5 c.c. from guinea-pig infected with *T. brucei*, containing 580,000 trypanosomes per c.mm.	0·815 c.c.	0·64 c.c.	0·05 c.c.	0·125 c.c.	0·255 c.c.	0·105 c.c.
5	A	3 c.c.	—	0·935 c.c.	0·62 c.c.	0·275 c.c.	0·04 c.c.	—	—
	B	,,	1·5 c.c. from normal rat	1·11 c.c.	0·755 c.c.	0·255 c.c.	0·07 c.c.	—	—
	C	,,	1·5 c.c. from rat infected with *T. brucei*, containing 270,000 trypanosomes per c.mm.	0·73 c.c.	0·645 c.c.	0·03 c.c.	0·055 c.c.	0·235 c.c.	−0·11 c.c.
6	A	3 c.c.	—	1·09 c.c	0·715 c.c	0·315 c.c.	0·06 c.c.	—	—
	B	,,	1·5 c.c. from normal guinea-pig	1·355 c.c.	0·96 c.c.	0·32 c.c.	0·075 c.c.	—	—
	C	,,	1·5 c.c. from guinea-pig infected with *T. brucei*, containing 800,000 trypanosomes per c.mm.	1·12 c.c.	0·95 c.c.	0·09 c.c.	0·08 c.c.	0·23 c.c.	−0·01 c.c.

TABLE 2—*continued*.

No. of experiment		Composition of Blood Mixture: Amount of defibrinated blood of normal rabbit	Composition of Blood Mixture: Amount of citrated plasma of normal or infected animal consisting of 2 parts plasma and 1 part citrated saline solution	Analysis of Gases obtained from Blood Mixture after Heating for 1 hour at 37° C. in the Absence of Air: Total	CO_2	O	N	Deficiency in amount of oxygen in C as compared with B	Variation in amount of carbon dioxide in C as compared with B
7	A	3 c.c.	—	1·205 c.c.	0·755 c.c.	0·385 c.c.	0·065 c.c.	—	—
	B	,,	1·5 c.c. from normal guinea-pig	1·38 c.c.	0·955 c.c.	0·32 c.c.	0·105 c.c.	—	—
	C	,,	1·5 c.c. from guinea-pig infected with *T. evansi*, containing 350,000 trypanosomes per c.mm.	1·11 c.c.	1·025 c.c.	0·01 c.c.	0·075 c.c.	0·31 c.c.	0·06 c.c.
8	A	3 c.c.	—	1·255 c.c.	0·82 c.c.	0·375 c.c.	0·06 c.c.	—	—
	B	,,	1·5 c.c. from normal guinea-pig	1·505 c.c.	1·08 c.c.	0·345 c.c.	0·08 c.c.	—	—
	C	,,	1·5 c.c. from guinea-pig infected with *T. evansi*, containing 45,000 trypanosomes per c.mm.	1·19 c.c.	1·085 c.c.	0·025 c.c.	0·08 c.c.	0·32 c.c.	0·005 c.c.
9	A	3 c.c.	—	1·325 c.c.	0·865 c.c.	0·36 c.c.	0·10 c.c.	—	—
	B	,,	1·5 c.c. from normal guinea-pig	1·68 c.c.	1·225 c.c.	0·37 c.c.	0·85 c.c.	—	—
	C	,,	1·5 c.c. from guinea-pig infected with *T. evansi*, containing 395,000 trypanosomes per c.mm.	1·23 c.c.	1·14 c.c.	0·05 c.c.	0·85 c.c.	0·32 c.c.	0·065 c.c.
10	A	3 c.c.	—	0·90 c.c.	0·42 c.c.	0·43 c.c.	0·05 c.c.	—	—
	B	,,	1·5 c.c. from normal rat.	1·40 c.c.	0·86 c.c.	0·45 c.c.	0·09 c.c.	—	—
	C	,,	1·5 c.c. from rat infected with *T. dimorphon*, containing 250,000 trypanosomes per c.mm.	1·32 c.c.	1·015 c.c.	0·22 c.c.	0·85 c.c.	0·23 c.c.	0·065 c.c.
11	A	3 c.c.	—	0·785 c.c.	0·355 c.c.	0·38 c.c.	0·05 c.c.	—	—
	B	,,	1·5 c.c. from normal rat.	1·105 c.c.	0·62 c.c.	0·39 c.c.	0·095 c.c.	—	—
	C	,,	1·5 c.c. from rat infected with *T. evansi*, containing 250,000 trypanosomes per c.mm.	0·875 c.c.	0·71 c.c.	0·08 c.c.	0·085 c.c.	0·31 c.c.	0·09 c.c.

TABLE 3. Data given in Table 2 re-calculated in volumes per cent.

No. of experiment		100 c.c. of mixture consisting of 2 parts defibrinated blood of normal rabbit and 1 part of citrated plasma from the following normal or infected animals	Analysis of Gases obtained from Blood Mixture after Heating in the absence of Air for 1 Hour at 37° C.					
			Total	CO_2	O	N	Deficiency in amount of oxygen in C as compared with B	Variation in amount of CO_2 in C as compared with B
1	B	Normal rat	22·2 c.c.	13·3 c.c.	6·1 c.c.	2·8 c.c.	5·5 c.c.	1·0 c.c.
	C	Rat infected with *T. equiperdum*. The citrated plasma containing 640,000 trypanosomes per c.mm.	16·6 c.c.	14·3 c.c.	0·6 c.c.	1·6 c.c.		
2	B	Normal guinea-pig	21·6 c.c.	12·7 c.c.	6·4 c.c.	2·4 c.c.	6·25 c.c.	2·3 c.c.
	C	Guinea-pig infected with *T. brucei*. The citrated plasma containing 340,000 trypanosomes per c.mm.	17·0 c.c.	15·0 c.c.	0·15 c.c.	1·8 c.c.		
3	B	Normal guinea-pig	25·3 c.c.	16·9 c.c.	7·0 c.c.	1·7 c.c.	7·0 c.c.	−4·0 c.c.
	C	Guinea-pig infected with *T. brucei*. The citrated plasma containing 450,000 trypanosomes per c.mm.	14·4 c.c.	12·9 c.c.	0·0 c.c.	1·6 c.c.		
4	B	Normal guinea-pig	20·2 c.c.	11·9 c.c.	6·8 c.c.	1·6 c.c.	5·7 c.c.	2·3 c.c.
	C	Guinea-pig infected with *T. brucei*. The citrated plasma containing 580,000 trypanosomes per c.mm.	18·1 c.c.	14·2 c.c.	1·1 c.c.	2·8 c.c.		
5	B	Normal rat	24·7 c.c.	16·9 c.c.	5·8 c.c.	1·7 c.c.	5·1 c.c.	−2·6 c.c.
	C	Rat infected with *T. brucei*. The citrated plasma containing 270,000 trypanosomes per c.mm.	16·2 c.c.	14·3 c.c.	0·7 c.c.	1·2 c.c.		
6	B	Normal guinea-pig	30·1 c.c.	21·3 c.c.	7·1 c.c.	1·7 c.c.	5·1 c.c.	−0·2 c.c.
	C	Guinea-pig infected with *T. brucei*. The citrated plasma containing 800,000 trypanosomes per c.mm.	24·9 c.c.	21·1 c.c.	2·0 c.c.	1·8 c.c.		
7	B	Normal guinea-pig	30·7 c.c.	21·2 c.c.	7·1 c.c.	2·3 c.c.	6·9 c.c.	1·4 c.c.
	C	Guinea-pig infected with *T. evansi*. The citrated plasma containing 350,000 trypanosomes per c.mm.	24·7 c.c.	22·6 c.c.	0·2 c.c.	1·7 c.c.		
8	B	Normal guinea-pig	33·4 c.c.	24·0 c.c.	7·7 c.c.	1·8 c.c.	7·1 c.c.	0·1 c.c.
	C	Guinea-pig infected with *T. evansi*. The citrated plasma containing 450,000 trypanosomes per c.mm.	26·4 c.c.	24·1 c.c.	0·6 c.c.	1·8 c.c.		
9	B	Normal guinea-pig	37·3 c.c.	27·2 c.c.	8·2 c.c.	1·9 c.c.	7·1 c.c.	− 1·9c.c.
	C	Guinea-pig infected with *T. evansi*. The citrated plasma containing 395,000 trypanosomes per c.mm.	27·3 c.c.	25·3 c.c.	1·1 c.c.	1·9 c.c.		
10	B	Normal rat	31·1 c.c.	19·1 c.c.	10·0 c.c.	2·0 c.c.	5·1 c.c.	3·5 c.c.
	C.	Rat infected with *T. dimorphon*. The citrated plasma containing 150,000 trypanosomes per c.mm.	29·3 c.c.	22·6 c.c.	4·9 c.c.	1·9 c.c.		
11	B	Normal rat	24·6 c.c.	13·8 c.c.	8·7 c.c.	2·1 c.c.	6·9 c.c.	2·0 c.c.
	C	Rat infected with *T. evansi*. The citrated plasma containing 250,000 trypanosomes per c.mm.	19·6 c.c.	15·8 c.c.	1·8 c.c.	1·9 c.c.		

Table 4. Analysis of gases obtained from defibrinated blood and from mixtures of this with plasma of infected animals before and after incubation at 37° C. for 1 hour in the absence of air.

No. of experiment		Composition of Blood Mixture		Analysis of Gases obtained from Blood Mixture before and after Heating for 1 hour at 37° C. in the Absence of Air					
		Amount of defibrinated blood of normal rabbit	Amount of citrated plasma of infected animal consisting of 2 parts plasma and 1 part citrated saline solution	Total	CO_2	O	N	Deficiency in amount of oxygen in C as compared with B	Variation in amount of carbon dioxide in C as compared with B
1	A	3 c.c.	—	0·83 c.c.	0·26 c.c.	0·52 c.c.	0·05 c.c.	—	—
	B	,,	1·5 c.c. from guinea-pig infected with *T. brucei* containing 37,500 trypanosomes per c.mm. Before incubation	1·16 c.c.	0·61 c.c.	0·48 c.c.	0·07 c.c.		
	C	,,	As B after incubation	1·07 c.c.	0·65 c.c.	0·35 c.c.	0·07 c.c.	0·13 c.c.	0·04 c.c.
2	A	3 c.c.	—	0·91 c.c.	0·43 c.c.	0·39 c.c.	0·08 c.c.	—	—
	B	,,	1·5 c.c. from guinea-pig infected with *T. evansi*, containing 100,000 trypanosomes per c.mm. Before incubation	1·13 c.c.	0·64 c.c.	0·36 c.c.	0·12 c.c.		
	C	,,	As B after incubation	0·82 c.c.	0·72 c.c.	0·03 c.c.	0·07 c.c.	0·33 c.c.	0·08 c.c.
3	A	3 c.c.	—	1·04 c.c.	0·42 c.c.	0·52 c.c.	0·09 c.c.	—	—
	B	,,	1·5 c.c. from guinea-pig infected with *T. brucei*, containing 35,000 trypanosomes per c.mm. Before incubation	1·27 c.c.	0·66 c.c.	0·52 c.c.	0·09 c.c.		
	C	,,	As B after incubation	1·17 c.c.	0·68 c.c.	0·39 c.c.	0·10 c.c.	0·13 c.c.	0·02 c.c.
4	A	3 c.c.	—	1·24 c.c.	0·68 c.c.	0·47 c.c.	0·09 c.c.	—	—
	B	,,	1·5 c.c. from guinea-pig infected with *T. evansi*, containing 287,000 trypanosomes per c.mm. Before incubation	1·27 c.c.	0·77 c.c.	0·41 c.c.	0·08 c.c.		
	C	,,	As B after incubation	1·11 c.c.	0·99 c.c.	0·05 c.c.	0·07 c.c.	0·36 c.c.	0·22 c.c.

TABLE 5. Data given in Table 4 re-calculated in volumes per cent.

No. of experiment		100 c.c. of mixture consisting of 2 parts defibrinated blood of normal rabbit and 1 part of citrated plasma of infected guinea-pig	Analysis of Gases obtained from Blood Mixture before and after Heating at 37° C. for 1 Hour in Absence of Air					
			Total	CO_2	O	N	Deficiency in amount of oxygen in C as compared with B	Variation in amount of carbon dioxide in C as compared with B
		Guinea-pig infected with *T. brucei*. The citrated plasma contained 37,500 trypanosomes per c.mm.						
1	B	Before incubation	25·8 c.c.	13·6 c.c.	10·7 c.c.	1·5 c.c.	2·9 c.c.	0·8 c.c.
	C	After incubation	23·8 c.c.	14·4 c.c.	7·8 c.c.	1·5 c.c.		
		Guinea-pig infected with *T. evansi*. The citrated plasma contained 100,000 trypanosomes per c.mm.						
2	B	Before incubation	25·1 c.c	14·2 c c.	8·0 c.c.	2·7 c.c.	7·3 c.c.	1·8 c.c.
	C	After incubation	18·2 c.c.	16·0 c.c.	0·7 c.c.	1·6 c.c.		
		Guinea-pig infected with *T. brucei*. The citrated plasma contained 35,000 trypanosomes per c.mm.						
3	B	Before incubation	28·2 c.c.	14·7 c.c.	11·6 c.c.	2·0 c.c.	2·9 c.c.	0·4 c.c.
	C	After incubation	26·0 c.c.	15·1 c.c.	8·7 c.c.	2·2 c.c.		
		Guinea-pig infected with *T. evansi*. The citrated plasma contained 287,000 trypanosomes per c.mm.						
4	B	Before incubation	28·2 c.c.	17·1 c.c.	9·1 c.c.	1·8 c.c.	8·0 c.c.	4·9 c.c.
	C	After incubation	24·7 c.c.	22·0 c.c.	1·1 c.c.	1·5 c.c.		

Apparatus.—The apparatus employed for this purpose was that illustrated by Figure I. The portion of the apparatus between Stop-cocks 1 and 2 was first filled with mercury. Through the two-way Cock 2 half a cubic centimetre of phosphoric acid (1 per cent. solution) was introduced into the vacuum Bulbs B to facilitate the removal of carbon dioxide. Cock 2 was now closed and a vacuum created in Bulbs B. The mixture of defibrinated blood and citrated plasma was then introduced by means of the receiver into Bulb A which communicated with the outside through Cock 1. Subsequently mercury was poured into the receiver and allowed to flow into the bulb until the blood just reached Cock 1, which was then turned so that the blood was now in contact with mercury both above and below. After allowing the mixture to react in Bulb A at 37° C. for one hour, Cock 2 was cautiously opened and the blood allowed to pass slowly into the vacuum bulbs. Cock 2 was then closed and the blood exhausted and the gases collected in Tube 1.

After complete exhaustion of the blood the gas collected in Tube 1 was measured and analysed by means of the apparatus indicated by figure 2.

Technique.—About 12 c.c. of blood was withdrawn from the external jugular vein of a normal rabbit and defibrinated by shaking with a few glass beads in a bottle. The blood was then filtered through gauze and placed in the ice-chest over night. The following morning it was thoroughly stirred and, after determining the percentage of haemoglobin present (in terms of human wet red cells), three samples each of 3 c.c. withdrawn and allowed to stand at room temperature, 10-12° C.

One of the three samples of defibrinated blood was then introduced into the apparatus in the manner described, and after warming to 37° C. for one hour in Bulb A was exhausted in the vacuum Bulbs B and the gases obtained measured and analysed.

To the second sample of defibrinated blood was added a definite volume of citrated plasma from an infected rat or guinea-pig containing numerous trypanosomes in suspension. (The number of trypanosomes per cubic millimetre in the citrated plasma was estimated by means of a Thoma Zeiss haemocytometer.) The mixture of blood and citrated plasma was then heated at 37° C. for

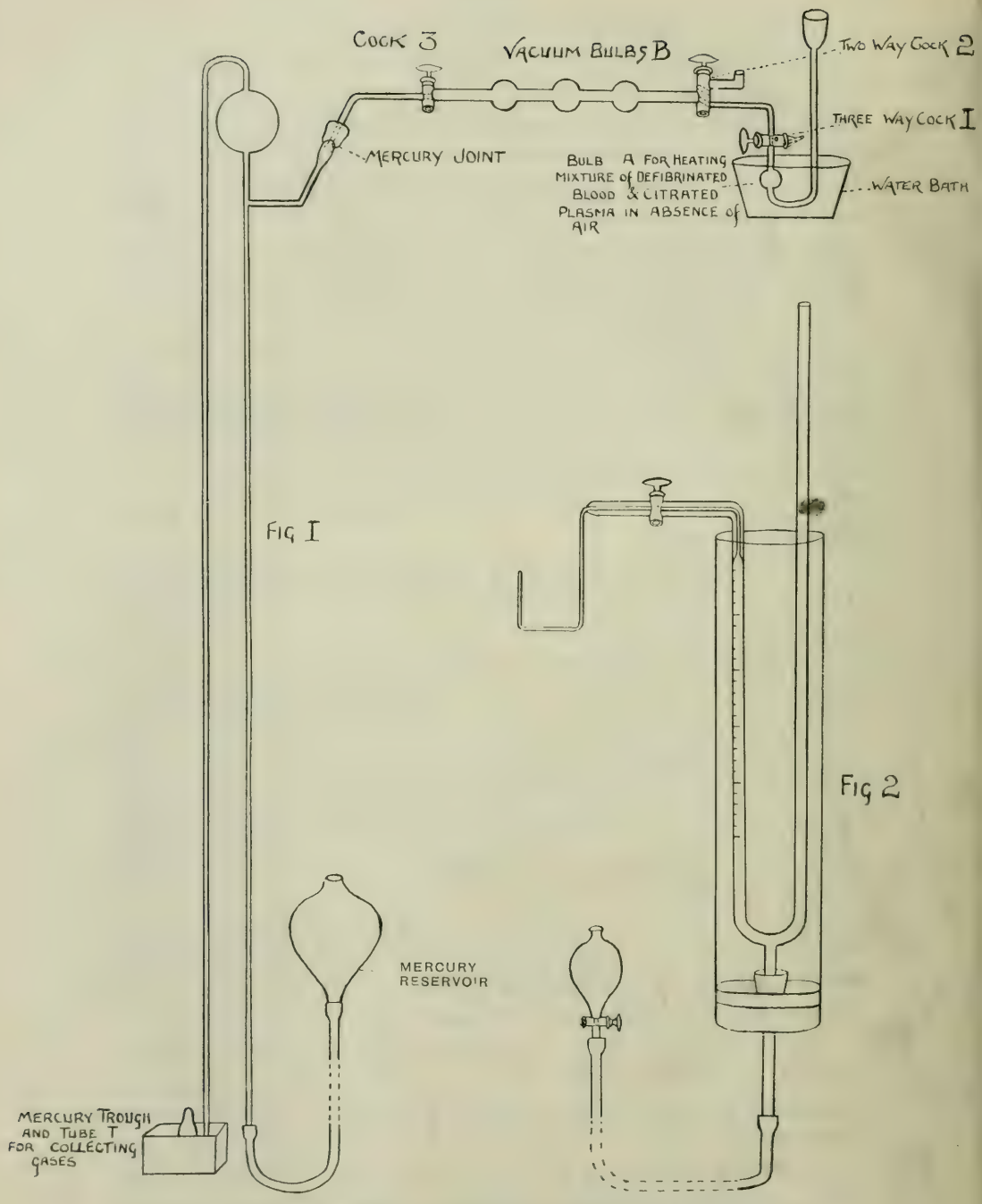


FIG. 1. Apparatus employed for incubating the suspension of trypanosomes and defibrinated blood in the absence of air, and for the subsequent removal of the gases contained in the blood mixture.

FIG. 2. Apparatus employed for measuring and analysing the gases.

one hour in Bulb A and the gases it contained subsequently determined.

A like volume of citrated plasma (diluted to the same degree as in the previous case) of a normal rat or guinea-pig was added to the third sample, which was then treated in the same manner as the other two cases.

The results obtained in these experiments are set forth in Table 2. It will be observed that in every case the action of the trypanosomes resulted in practically complete disappearance of oxygen from the gases subsequently exhausted from the mixture of defibrinated blood and infected plasma. A second point of interest is that the amount of carbon dioxide was not increased in a degree corresponding to the diminution of oxygen.

In Table 3 the results given in Table 2 are re-calculated in volumes per cent.; or, in other words, the quantity of gas present in 100 c.c. of the mixture—defibrinated blood plasma—estimated.

In a second series of experiments the procedure was similar, except that instead of employing as controls the plasma of a normal animal that of the infected animal itself was used. Here, however, reaction was not permitted to proceed in Bulb A of the apparatus, but the blood and plasma were passed straight on into the vacuum bulbs and immediately exhausted.

As will be seen from Tables 4 and 5 the results obtained are similar to those of the first series of experiments.

Although actively motile trypanosomes cause so considerable a reduction of solutions of methylene blue and haemoglobin *in vitro*, yet the mere presence of numerous parasites in the blood of the living organism is of itself insufficient to give rise to a purple condition of the blood. The blood of rats swarming with parasites is generally of the normal red colour. Under these circumstances the oxygenation of the haemoglobin occurring in the lungs is sufficient to counterbalance the reduction resulting from the action of the parasites.

As we have already mentioned, the purple appearance is most frequently to be observed in the blood of rabbits in a late stage of the disease. In these animals trypanosomes are usually absent from the peripheral blood or present in small numbers only. All animals presenting the phenomenon had, however, marked involvement of

the respiratory passages and the breathing was stertorous and laboured. The external nares was often almost completely obliterated by an oedematous and infiltrated condition of the skin and mucous membrane. Post mortem examination showed extensive thickening of the mucous membrane of the nose and pharynx, and sometimes even of the trachea and larger bronchi. Sections of the affected tissues usually revealed the presence of trypanosomes often in very considerable numbers.

SUMMARY

The blood of certain animals in the later stages of trypanosomal infections is frequently of a dark purple colour. This appearance results from deficient oxygenation of the haemoglobin.

Living trypanosomes cause marked reduction of solutions of methylene blue and also of those of oxyhaemoglobin.

The incubation, in the absence of air, of living trypanosomes in defibrinated blood of a normal animal causes considerable reduction or—if the parasites be numerous—total disappearance of the oxygen combined with the haemoglobin. A corresponding increase in the amount of carbon dioxide has not been found.

The mere presence of numerous parasites in the peripheral circulation is not, however, sufficient to account for the purple colour of the blood, since the blood of rats and guinea-pigs swarming with parasites is usually of the normal bright-red appearance.

The purple colour is most marked in the blood of rabbits in the later stages of the disease. Although the peripheral blood of these animals does not contain large numbers of parasites, yet the respiratory passages are found to be considerably involved. The nasal mucous membrane and that of the trachea and bronchial tubes is often extremely oedematous and infiltrated, and the respiration of the animal is stertorous and laboured. On microscopical examinations of sections of these tissues trypanosomes were often found in large numbers in the oedematous mucous and sub-mucous tissue.

THE ANTI-MALARIAL OPERATIONS AT ISMAILIA

BY

J. W. W. STEPHENS, M.D. (CANTAB.)

(*Received for publication* 26 *April*, 1911)

That the Anti-malarial operations at Ismailia following on the report of Sir Ronald Ross (1903) have resulted in completely freeing the town from Anophelines and hence from malaria is a well-known fact, but I do not think it is equally well known what exactly was done at Ismailia to secure this result. I have attempted here to give as complete an account as possible; the only existing account so far being the brochure in French of the Suez Canal Company, of which I have made full use*†. I have thought it of interest also to attempt to trace historically the beginnings of malaria at Ismailia.

I. HISTORICAL

The history of malaria in the Isthmus of Suez and more particularly at Ismailia is bound up with the history of the water supply of the region. The problem of supplying water to the workmen engaged in cutting the Suez Canal through the desert, was always a pressing one in the early days of the construction. The three sources of water that were at successive periods available were, viz., (1) the wells, (2) the alimentary canal, and (3) the freshwater canal which flows from Cairo to Ismailia.

THE WELLS

1859. April 25. The work of constructing the Suez Canal was commenced.

1859-1860. Water was found at the following sites near Ismailia, the centre of the Isthmus.

* While this article was in the press a paper by Bruce (1911) has appeared dealing with the subject from rather a different standpoint.

† I beg to tender here my grateful thanks to Dr. Pressat and Dr. Cambouliu of the Suez Canal Company for much information kindly supplied.

1. *El Ferdann.* Water was found at a depth of 2·50 metres, but it was salt.

2. *Saba'h 'Byar* (Seven Wells) in Wadi Tumilat.

3. *Bir Abu Balah.* Wells existed here from Biblical times. Six other pits dug gave only salt water.

4. *Lake Timsah.* Workmen were employed in cutting the reeds at the foot of the Nefisha sand-dunes, and the tamarisks bordering the lake, showing that the soil contained sufficient moisture to support vegetation.

5. *Fawar.* Passable water found at 3·30 metres. This was the site of an ancient pit.

6. *Tusum* (plateau). Abundant but slightly brackish water found at a depth of 13 metres. Previous to finding water here, it was conveyed by dromedary from Awebet Station, six hours' distance from Timsah.

1860-1861. Water in the centre of the Isthmus (Lake Timsah) was procured from wells sunk at the following places.

7. *Bir Abu Balah.* Water was found at a depth of 4·70 metres. This water was distributed by a water-wheel used for irrigating the attempts at cultivation of barley, cotton, melons, haricots, etc., over an area of 14,200 square metres. In October 6,400 square metres were ready to be sown. It is interesting to note, that at this time even, possibilities of pool formation existed, as very probably was the case with uncontrolled irrigation.

8. *Tusum.* Wells were dug, and near them a layer of clay, 1·20 metres thick, was found. A small garden, 30 by 20 metres, was planted with barley, wheat, bersim, cauliflowers, onions, haricots, sea-kale, etc.

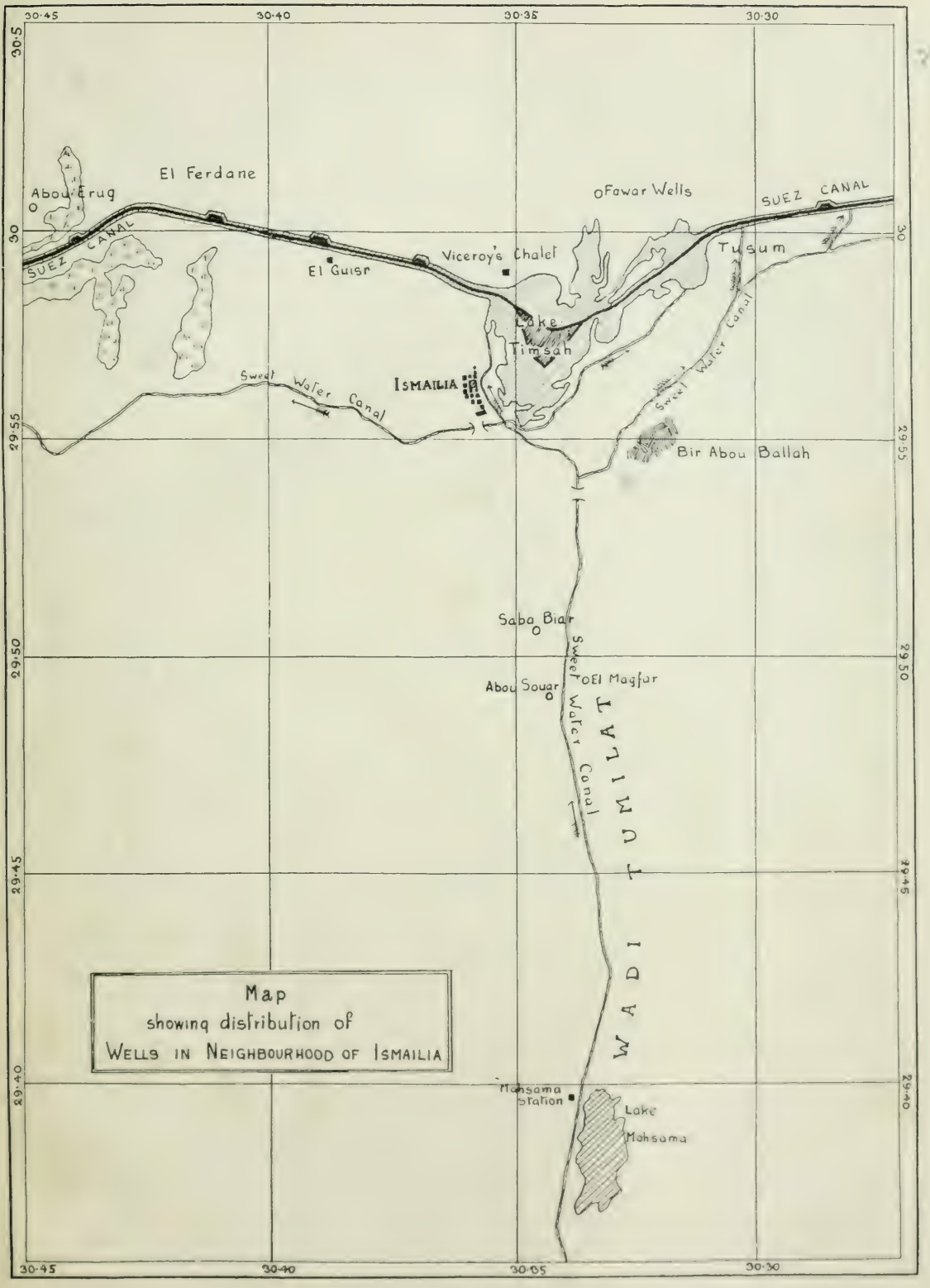
9. *Serapeum.* Water was found at a depth of about 17 metres.

10. *Abu Souer*, near Makfar, 15 miles from Seuil (El Guisr). These wells in the Wadi Tumilat Valley furnished excellent water.

11. *Nefisha.* Brackish but drunk by animals.

12. *Saba'h 'Byar.* Three miles from Makfar, close to Nefisha, gave sweet water. 'Excellent water throughout the year.' (Anonymous, 1857).

13. *Abu Erouq.* East of the Suez Canal, near Working-camp I, brackish but drunk by animals.



Map
showing distribution of
WELLS IN NEIGHBOURHOOD OF ISMAILIA

Alimentary Canal.

In order, however, to secure a more abundant supply of water for the working camp at El Guisr, about six miles north of Timsah (Ismailia), water was got from Lake Mahsama (average depth $\frac{2}{3}$ metre), in Wadi Tumilat.

A water-course, 26,800 metres long, 0·30 metres broad at bottom and 1 metre at water level, was constructed as far as Bir Abu Balah, where it was collected in a masonry reservoir. From here it was brought in pipes to Timsah (Ismailia) to a well and pumped up to a 64 square metre sheet-iron tank; from here, again, it was taken in earthenware pipes to El Guisr, where a second pump and reservoir was again used for distributing it as far as El Ferdann (about eight miles from Ismailia). The workmen digging this water-course got their water by camel from Nefisha. During a temporary failure of this supply, water was carried by camels from the sites (10-13) just mentioned.

Freshwater Canal

1862. Reached Timsah (Ismailia), and after the 23rd January was opened for navigation. It comprised (1) an old canal from Zagazig to Qassasin, and (2) a portion from Qassasin to Ismailia, 7·70 metres wide.

Sluices were constructed at Nefisha to carry off the excess of water into the lagoons bordering Lake Timsah. The difference of level between the freshwater canal and the Suez Canal was 6·6 metres, necessitating the intervention of locks.

1862. April 27. The first stones of the foundation of Ismailia (originally Timsah) were laid (though in 1862 two or three chalets had been begun), streets were laid out, palms planted. The population in September, 1862, was:—Europeans, 150; Arabs, 593.

(1863. Timsah called Ismailia.)

1865. A new canal in lieu of the old one built from Abassa to Qassasin, 10 metres wide.

1866. July. The canal termed the Ismailia Canal.

(1869. 17 November. Suez Canal opened.)

1870. The portion of the canal from Abassa to Qassasin enlarged to 13 metres.

1874. Portion from Qassasin to Ismailia enlarged from 7·70 metres to 13 metres (commenced).

1877. The new canal inaugurated, 15 April.

1896. Hydraulic rams used for distributing the water at Ismailia, previously it was done by the pumping station (*usine des eaux*) at Ismailia.

Circle Canal

1863. July-August. A pumping station was established at the extreme East of the town, to supply the stations between Ismailia and Port Said. This was supplied by a branch of the sweet-water canal, starting 720 metres above the upper lock at Ismailia. It was 2,670 metres long, and followed the North of the town, 0·5 metre wide at bottom, depth 0·75 metre. It furnished *water for the cultivations established along part of its course* and for a large *experimental garden* around the pumping station.

1866. It was cleaned.

1877. It was considerably enlarged at the same time that the Abassa-Ismailia Canal was replaced by one of larger section.

1880. It was filled up and dried.

These data are, I think, sufficient to show that breeding places existed from the earliest times, and that Anophelines also existed will be shown in the next section, hence I consider that the view of E. H. Ross (1909), that malaria came to Ismailia in 1877 with the enlarged freshwater canal, is incorrect.

II. ORIGIN OF MALARIA

1861. 'Among the numerous workmen employed at the construction of the freshwater canal, there occurred nine cases of simple intermittent fever which yielded easily to quinine. They had contracted the malady on the borders of Lake Mahsama, where the *fever showed itself each year, especially* after the rise of the Nile.' Voisin Bey (1906).

I conclude from this that malaria was, even at this time, endemic in the valley of the freshwater canal, and Anophelines also must have existed in the region.

1865. 'The town (Ismailia) was extensively watered by a system of pipes which conducted the water into each dwelling and permitted the establishment there of vegetable and flower gardens. The sanitary condition was normal in January, but in February ordinary illnesses began to take more grave forms, especially among the new arrivals—Calabrians, Dalmatians, Bretons and Greeks. Illnesses begun in Europe, and unknown in the works-stations on the Isthmus, showed themselves. Pernicious fevers, fever of a remittent type, etc., occurred. The mortality was 2·5 per cent.' Voisin Bey (1906).

1866. 'Among some work-stations, among others El Guisr (about three miles from Ismailia), cases of simple intermittent, and some cases of pernicious fever occurred. The Isthmus had, up to this time, been free from this malady, but this year the fevers had been fairly frequent, and had assumed the paludic character without being otherwise dangerous or obstinate. There was a kind of general paludic influence.' Voisin Bey (1906).

It seems clear from this that malaria was fairly common in the Isthmus, and that it is inconsistent to say that 'the Isthmus had, up to this time, been free from this malady.' We see thus early the seeds sewn of the crop which was eventually gathered in Ismailia.

After this date we have no further records, but it is hardly credible that no more malaria occurred until the outbreak in Ismailia in 1877. We believe rather that malaria was always endemic in the Lake Mahsama region, that malaria and Anophelines spread from there. Probably Anophelines were always present in the Nefisha lagoons as they were in 1908. (Three cases of malaria in 1905, Bulletin (1909), p. 8.) No doubt also many cases were European in origin.

1877. Epidemic. Pressat (1905), attributes the outbreak to the fact that the enlargement of the freshwater canal to 13 metres led to a superabundant water supply, which, filtering through the sand, formed a subterranean layer which formed pools at every suitable spot, that subsequently Anophelines were introduced by boats along the sweet-water canal, or by rail. The increase in water very likely led to more pools in this way, but pools and Anophelines must have existed long before this, as the freshwater canal reached Ismailia as early as 1862, and irrigation was proceeding in 1863,

and, moreover, the sub-soil water was always present, making itself apparent especially at high Nile, and, as a matter of fact, as we have just seen, malaria was recorded at Lake Mahsama in 1861, and at Ismailia itself in 1865. The data now available are, however, insufficient to explain, with certainty, why malaria, which we believe always to have been endemic at Ismailia, became epidemic in 1877 (300 cases). Nor, again, is the cause of the rise in 1886 (2,500 cases) explained.

III. THE ANTI-LARVAL OPERATIONS

Ismailia is situated on the North bank of Lake Timsah (Lake of Crocodiles). The lake received at rather high Niles fresh water (reaching it through the Valley of Gessen, roughly at right-angles to the Suez Canal, and through which region the present freshwater canal flows) according to an observer (Anon., 1857), who also states that he found the muddy sediment of the Nile in its swamps. Before the cutting of the Suez Canal, the water in it was intensely salt (owing to the underlying bed of salt); its depth was only 0·6 metre, but along its margin reeds grew in abundance and tamarisks (Anon., 1857) also occurred, deriving their nourishment (presumably) from the underlying sub-soil water.

Over a great part of the Isthmus, according to Roux (1901), one finds in the sub-soil a layer of water scarcely brackish, which is held in the sand by a thin layer of clay, and which flows about at the level of the sea and that of the Suez Canal.

Boyce (1904), notes: 'freshwater grasses, and other freshwater plants, growing along the margin of the canal, and even in the water of the canal itself, which is strongly salt. The explanation given to me of this occurrence was that the sub-soil fresh water in the bank of the canal afforded the necessary moisture for the roots.'

Ross (1903, p. 5), states that 'the sub-soil water is very near the surface and, as we are informed, fluctuates with the rise and fall of the distant Nile. In some spots near Ismailia, where the surface of the desert is much depressed, this sub-soil water produces considerable lakes and ponds, but owing to the extreme salinity of the sand most of these pools are brackish, their shores being encrusted with salt, supporting but little vegetation. There are

several spots, however, where the water is nearly, if not quite fresh, and here we observe a considerable amount of cultivation—grass and vegetation. There are even places where the fresh, natural waters produce shallow marshes of small extent; where small pools form among reeds and grass. And these can be found not only close to Ismailia but, as I was informed, in many parts of the desert, and can be seen along the railway to Cairo. But it must be understood that such areas are very small in extent when compared with the large surface of perfectly arid sand which surrounds the town.'

Breeding-Places of Anophelines and Anti-Larval Measures Adopted

Ross (1903, p. 12) says that 'whenever we examined the marshes connected with the natural waters which exist around Ismailia, we succeeded in finding numerous larvae of Anopheles and also of Culex. The insects existed especially among the short grass and other vegetation growing on soil covered with a very thin layer of water. On one series of pools situated to the East of the town, we found innumerable larvae both of Anopheles and Culex existing in water which was so brackish as to contain nine grams of salt per litre.'*

He sums up the breeding-places as follows:—

1. The small marshes in the midst of the cultivation to the East of the town (? Abu Rahan).

2. The still smaller marsh close to the abattoir. [(o) on map.]

3. A few were observed in an artificial fountain in the middle of the European station.

The following account is based on the data contained in (1) Pressat, 'Le Paludisme et les Moustiques' quoted as (P.) and (2) 'Suppression du Paludisme à Ismailia,' Compagnie universelle du Canal maritime de Suez, quoted as (S.), and on personal communications from Drs. Pressat and Cambouliu.

Pressat (p. 132) describes the Abu Rahan marshes as being infested with Anophelines, and that work was carried on there in

* These Anophelines may have been *Pyretophorus cleopatrae* Wilcocks, an Egyptian species which commonly breeds in saltish water, whereas *Cellia pharoensis* does not. Whether *P. chaudoyei*, an Algerian species, also exists in Egypt is, I think, doubtful.

'clouds of Anophelines' (p. 132). Elsewhere he describes the breeding places as being 'pools, drains, camel foot-prints in the environs of the town' (p. 129).

Abu Rahan Marsh.*

This marsh is situated to the North-east of the town, less than a mile (1,640 yards) away. It has an area of between nine and ten acres (44,252 square yards) and has an average depth at the portions marked A and B of four and a half feet and a maximum of about eight feet.

Presence of Larvae.

1901. August. Many were found in the irrigating channels that supplied the plantations of this marsh.

1902. September. Ross's data probably apply to this marsh.

Destructive measures.

1880-1881 or 1885. This marsh had been partly filled up in these years and planted with Casuarina, Eucalyptus and Palms. It had also been drained by a channel leading to the lake, but this drainage had been ineffective for a forest of reeds had grown over the whole of the Northern area. Moreover, at the rise of the Nile (in the Autumn) all the inequalities of the soil filled with water and especially the irrigating channels along the bases of the trees.

Note.—These preliminary efforts were made before the mosquito as the malaria agent was known.

1903. (*a*) The main drain was deepened and cleaned throughout so as to maintain a proper fall to the lake. The fall of the drain was 0·0005 per metre.

(*b*) Two encircling drains and three cross drains were constructed.

(*c*) Further, sluices were constructed so as to dam up the water, and subsequently to flush out the channels if necessary, but this has not been required.

(*d*) The reeds were cut everywhere.

(*e*) The soil was carefully levelled by filling up all depressions and all the irrigation channels.

---

*I have been unable to ascertain when this marsh first came into existence.

Workmen were assigned to keep the drains at the proper depth and to keep their banks free from vegetation, and also to keep the marsh absolutely free from reeds.

Result.

1903. August. No larvae could be found either in the contributory channels or in the main drain. It must be noted that all these channels and the main drain contained fish, viz., *Mugil cephalus*, *M. capito* and *M. seheti* and also *Tilapia gallilea* (Arabic Chaba'r). Whether these fish destroy larvae is unknown, but *T. gallilea* will at least destroy them rapidly when hungry in an aquarium (P.).

The Subsidiary Marshes.

(1) Marsh H.

To the East of Abu Rahan is of slight depth.

Presence of larvae.

Along the margins there were numerous Anopheline pools.

Destructive measures.

It was filled up (with sand).

(2) Marsh I.

To the North-West of Abu Rahan, has an area of 22,962 square yards, equal to 4-5 acres, and a depth of about 2 feet 9 inches.

Presence of larvae.

No records.

Destructive measures.

It was filled up and united to the drains of Abu Rahan by a subterranean channel.

(3) Marshy Ground along Lemasson Avenue.

Pools formed during the rise of the Nile.

Presence of larvae.

No records.

Destructive measures.

1903. These had been filled up many years before, but they were now re-covered with a sufficiently thick layer of sand.

Southern Region.

Small depressions near the North bank of the lake, which contained water at high Nile.

Presence of larvae.

A few.

Destructive measures.

They were filled up with sand.

Western Region.

From the outskirts of the town to Nefisha Station, a distance of more than 5 kilometres (3 miles), exists a stretch of land lying between the sweet-water canal and the lake. The level of the sweet-water canal along this district is 6 metres (19 feet 8 inches) above the mean level of the lake. This land, like the rest of the desert when irrigated, is fertile and is let out to cultivators, but it is absolutely necessary for cultivation purposes that the water should not lie stagnant but be drained away. For this purpose the cultivators had constructed a number of canals but at the time of the anti-malaria campaign they were badly kept, full of weed and almost stagnant. We may consider these in more detail.

Western Region (N).

At this point there existed numerous small puddles and marshy areas covered with grass or bulrushes.

Presence of larvae.

Present, a dangerous focus (S. p. 15).

Destructive measures.

Filled in with sand.

Western Region (O, Q).

At the points O and Q hydraulic rams exist for the supply of water to the town and hospital respectively. The drains in connection with O led into the lake, while that of Q ran into Nefisha lagoon, an arm of the lake. Both were in a state of bad repair, the gradients were imperfect so that water remained stagnant in them, the banks had broken down, and water-cress beds had been established along their margins.

Presence of larvae.

Abundant, 'a dangerous focus' (S. p. 15). Ross also cites the marsh existing close to the Abattoir (O) as containing larvae.

Destructive measures.

The drains were cleaned, deepened, the banks repaired, the cress-beds done away with, all vegetation removed. The drain of hydraulic ram O was divided into branches, the eastern branch draining the neighbouring cultivated land.

CULTIVATED AREAS R, S, T, U.

The drains in these areas were exceedingly numerous.

Presence of larvae.

Abundant, 'a dangerous focus' (S. p. 15).

Destructive measures.

Numerous old drains were replaced by one large drain traversing a dune and discharging into Timsah. In other cases the drains were repaired, deepened and the banks kept in good order.

Result.

The result of these works on the cultivated lands was not at first satisfactory, for Anophelines still continued to breed in the gutters of the low-lying marshy parts opening into the lagoon, where the water was shallow and almost stagnant. Accordingly the plan was devised of damming (weekly) the irrigation water that supplied these areas. As the freshwater canal is about twenty feet above the lake level there is produced, on opening the dams, a very rapid and powerful stream, sufficient to sweep out all larvae. The result of this procedure was completely satisfactory. (This method of damming the water is now practised in all the irrigation channels. The water is led into those areas that require irrigation at definite intervals. It is then shut off, the result being that the water supplied to the area in question has all sunk into the ground in two or three days).

Result.

From the summer of 1903, no larvae found in the whole of the protected area* (S. p. 20).

* It is not clear whether the areas O, P, Q, R, S, T, U, were at this time included in in the term 'protected area' for in a letter Dr. Cambouliu informed me that the areas O, P, Q, R, S, T, U, were first drained in 1904-1905. In a subsequent letter, however, he states that 1903 is the correct date.

MARSH I.

This is situated to the West of Nefisha, is deep and contains several species of fish.

Presence of larvae.

None (Dr. Cambouliu).

Destructive (?) measures.

1906. Drainage commenced. Now completed, 1910.

MARSH V., SOUTH OF NEFISHA LAGOON.

Larvae.

Along the shore at the entrance of the drains. The drains contain numerous fish.

Measures.

1898. Levelled, planted and drained. 1903 (?), the pools containing larvae, filled with sand.

OTHER EXTENSIVE MARSHES.

Exist along the course of the sweet-water canal. They derive their water from the great drain, Bir Abu Balah, beyond Nefisha, but their drainage would be extremely costly.

ADULT ANOPHELINES.

Very few data exist as to their numbers in Ismailia, but it is stated (S. p. 11) that the whole town was invaded, and elsewhere (p. 16) that they appeared each year precisely in the autumn, but in regard to the first statement in a private communication Dr. Pressat states: 'certainement très peu parmi les grands quantités d'autres moustiques.'

In Abu Rahan marsh, adult mosquitos in large quantity (S. p. 15).

OILING OF POOLS.

Besides treating the culicine breeding grounds the brigade also oiled 'toutes les mares, rigoles, flaques d'eau, les pas de chameaus, des jardins et des alentours de la ville' (P. p. 134).

This, Dr. Pressat informed me, refers to the small collections of water in and around the town, and not to the marshes such as Abu Rahan.

Summary

As stated above, in August, 1903 (?1904, 1905), there were no larvae in the whole of the protected area, hence *ipso facto* from this time all fresh cases of malaria must have ceased, so that the anti-larval measures were a complete success. Apart from the drainage as a whole, I consider that the following three factors played an important part in achieving this success, viz.:—(1) Ismailia is in the desert; (2) the intermittent irrigation system with a fall of 20 feet from the freshwater to the maritime canal; (3) the presence of fish in all the drains.

Cost of the Operations

(1) *Initial expenses*, £2,000. This was incurred in filling up swamps and drainage (S. p. 25). Presumably this is up to 1906, the following data extend to 1909.

(2) The non-recurrent expenses incurred for filling up the pools and drainage of the swamps and arable lands situate in the neighbourhood of the town, and to which the Egyptian Government has contributed a part, to-day (1909) reach a total of about 100,000 francs (£4000). The area of the improved land represents about 400 hectares (roughly 1,000 acres), so that the cost was £4 an acre (Bulletin 1909).

(1) *Permanent expenses.* In keeping drains in good repair cutting reeds and flushing drains, £312 per annum. This does not include the sum spent in anti-Culicine work, which we have not considered here (S. p. 25). To this would also have to be added the expense of oiling small collections of water—possible Anopheline breeding places.

(2) The permanent expenses for disinfection, the hunt for mosquitos and larvae, and the maintenance of the improved lands have remained at about 18,000 francs (£720) per annum since 1903 (Bulletin 1909). This, no doubt, includes expenses of Culicine destruction, and accounts for the discrepancy between the two statements.

(3) The following table is added for the sake of completeness, though it does not appear that the expenses can be fairly put solely

to the account of anti-malarial measures. In fact, here, as in other figures, no details are given, and in this case it is impossible to say what proportion must be ascribed to each of the three headings.

Wages paid to fever patients whilst not working. Curative medicines distributed to old malaria patients. General prophylactic measures*.

TOTAL OF EXPENSES

1903	38,209	francs	£1,528	approximately
1904	25,986	,,	1,039	,,
1905	17,420	,,	696	,,
1906	16,963	,,	678	,,
1907	15,642	,,	625	,,
1908	16,806	,,	672	,,

IV. THE QUININE PROPHYLAXIS

1902. February. This was instituted at this time, '*à tout le personnel de la Compagnie* qui en a retiré un grand bénéfice' (P. p. 111), but I understand privately from Dr. Pressat that it was optional in the case of employés (about 2,000) and obligatory in the case of workmen (about 7,000). The latter, for example, if sick, lost a part of their salary, otherwise paid to them, unless they had taken their quinine in the fixed dose, in the presence of the overseers (S. p. 9). The Company, in fact, were in a position to ensure their orders being carried out.

Prophylactic dose.

This was given in the form of two pills of 1½ grains each for three consecutive days, then a week's interval in the first half of the year, and a three day interval in the latter half of the year (the 'fever season') (S. p. 10).

* Ross (Bulletin 1909, p. 4) states 'I am informed that a considerable part of these expenses have been and are incurred not only for malaria prevention but also for agriculture and other purposes.' This is another illustration of the difficulty of finding out what exactly the published data connote.

Result.

As an example, it is stated (P. p. 106) that 'ces nombreuses équipes (centaines des ouvriers)' receiving daily, in this case, 3 grains of quinine, 'travaillèrent plusieurs mois,' in Abu Rahan marsh, 'dévoreés par les Anopheles et pas un des ouvriers ne prit la fièvre'; while, 'à lá même époque une compagnie particulière qui exécutait dans la même région des travaux de dragage sur le canal Abassieh, et qui ne prenait pas les mêmes précautions vit ses équipes litteralement fondre sur les atteintes de la malaria' (microscopically confirmed). Here it is not stated that these latter workmen were devoured by Anophelines, so that the difference might be attributed to their absence. If we are to assume that the first set of men were infected in the marsh then the mosquitos must have been infected there by gamete-carriers among the men, so that we get a *marsh* full of '*infected*' mosquitos. If such a condition existed it is an interesting and certainly an exceptional one, but it is, I think, more probable that these workmen, like the other gang, were infected in their houses, the difference between the two cases being the quinine prophylaxis.

It should be stated, however, that, according to Pressat (p. 132), Abu Rahan was formerly so unhealthy that one could not undertake the least work without redoubling the malaria. Elsewhere (P. p. 130) it is stated that circulars were issued. The employés and workmen were informed that there would be a daily gratuitous distribution of quinine. They were asked at the same time to suppress water in their 'bassins d'arrosage' and to suppress all stagnant water (P. p. 130).

Result.

This was appreciable, the number of malaria cases being:—

1901	...	...	...	...	1990
1902	...	...	...	...	1551

We have no data, however, as to what proportion of these cases were employés for whom the prophylaxis was optional.

Similarly in the dispensary we find a peculiar but more marked fall, the figures being:—

1900	...	2,591	1903	...	6
1901	...	476	1904	...	1 (first six months)
1902	...	85			

From a private communication from Dr. Pressat I learn that in 1901 he began systematic blood examinations of the dispensary

patients, nearly all natives, so that the figure 476 may be accepted as accurate for 1901. We have then, in 1902, the great fall to 85. This fall is, I think, certainly due to the quinine prophylaxis of 1902 (for it was in December, 1902, that it was first decided systematically to undertake anti-larval measures after Major Ross's visit in September, 1902), for as we have seen the prophylaxis was compulsory on the labourers. (This fall must have had, too, some effect on the hospital statistics, for presumably some of these cases would become hospital patients.) To what the extraordinary fall of 2,591 in 1900 to 476 in 1901 is to be attributed is not clear. For although diagnosis made by the 'sisters' of the dispensary may not be very accurate, yet if these 2,591 cases of 'fever' were not malaria, we are at a loss to what to ascribe them, for, as Ross states, 'without it (malaria) Ismailia would be a settlement almost free from infectious diseases.' It should be added that quinine at this time was given by the 'sisters' to those who came to ask for it, but to what extent it was given, what effect it had it is impossible to say, unless one accepts these figures as affording the answer.

1903. The quinine prophylaxis was continued and in the pre-epidemic months the supervision was more strict. Circulars were issued explaining how to take it. It was distributed freely in the office, workshops and at the dispensary.

The following figures (P. p. 138), indicate to what extent the compulsory prophylaxis was carried out:—

QUININE PROPHYLAXIS.

1902 (9 months)	... 9,900 francs	...	£396
1903	... 14,780 ,,	...	591·2
1904	... 14,214 ,,	...	568·56

The following data differ again from these, but are official.

INTERNATIONAL CO-OPERATIVE PHARMACY AT ISMAILIA.
SALE OF QUININE.*

1902	... 16,905 fr. 10	... £656·20	approximately.
1903	... 15,678 fr. 20	... 627·12	,,
1904	... 11,624 fr. 17	... 464·96	,,
1905	... 6,482 fr. 46	... 259·28	,,
1906	... 6,689 fr. 90	... 267·56	,,
1907	... 8,821 fr. 96	... 352·84	,,
1908	... 5,927 fr. 44	... 237·08	,,

* Bulletin 1904, p. 9.

It is impossible then to estimate definitely the result of the quinine prophylaxis owing (1) to the incompleteness of the data, and (2) to the fact that in 1903 it was complicated by the anti-larval measures. We have, however, quoted some results that must be attributed to it. Whether malaria would have been stamped out at Ismailia by the quinine prophylaxis it is impossible to say. Had not anti-larval measures been adopted in 1903 then we would have had a quinine prophylactic experiment carried out under ideal conditions, and the result would have been of great scientific interest.

If, however, the Areas O, P, Q, R, S, T, U, were not drained till 1904-1905* it is probable that the decrease *in malaria* in 1903 was due in considerable part to the quinine prophylaxis, though the same result would have been arrived at without the taking of a grain of quinine.

NOTE.—The map is adapted from that published by the Suez Canal Company.

LITERATURE

Anonymous (1857), 'Isthmus Suez Ship Canal, etc.' London, John Weale.

— (1906), 'Suppression du Paludisme à Ismailia.' Compagnie universelle du canal maritime de Suez, Paris.

Bulletin (1909) of the Liv. School of Trop. Med., No. 1. Liverpool.

BOYCE, Sir RUBERT (1904), 'The Antimalarial measures at Ismailia.' Liv. School of Trop. Med. Memoir XII, p. 5.

BRUCE, Sir DAVID (1911), 'Report on the present conditions of Ismailia as regards malarial fever.' Journ. of the Roy. Army Med. Corps, p. 402.

PRESSAT, ANDRÉ (1905), 'Le Paludisme et les moustiques (Prophylaxie).' Paris, Masson et Cie.

ROSS, E. H. (1909), 'The Prevention of fever on the Suez Canal.' Cairo.

ROSS, RONALD (1903), 'Report on Malaria at Ismailia and Suez.' Liverpool School of Trop. Med. Memoir IX.

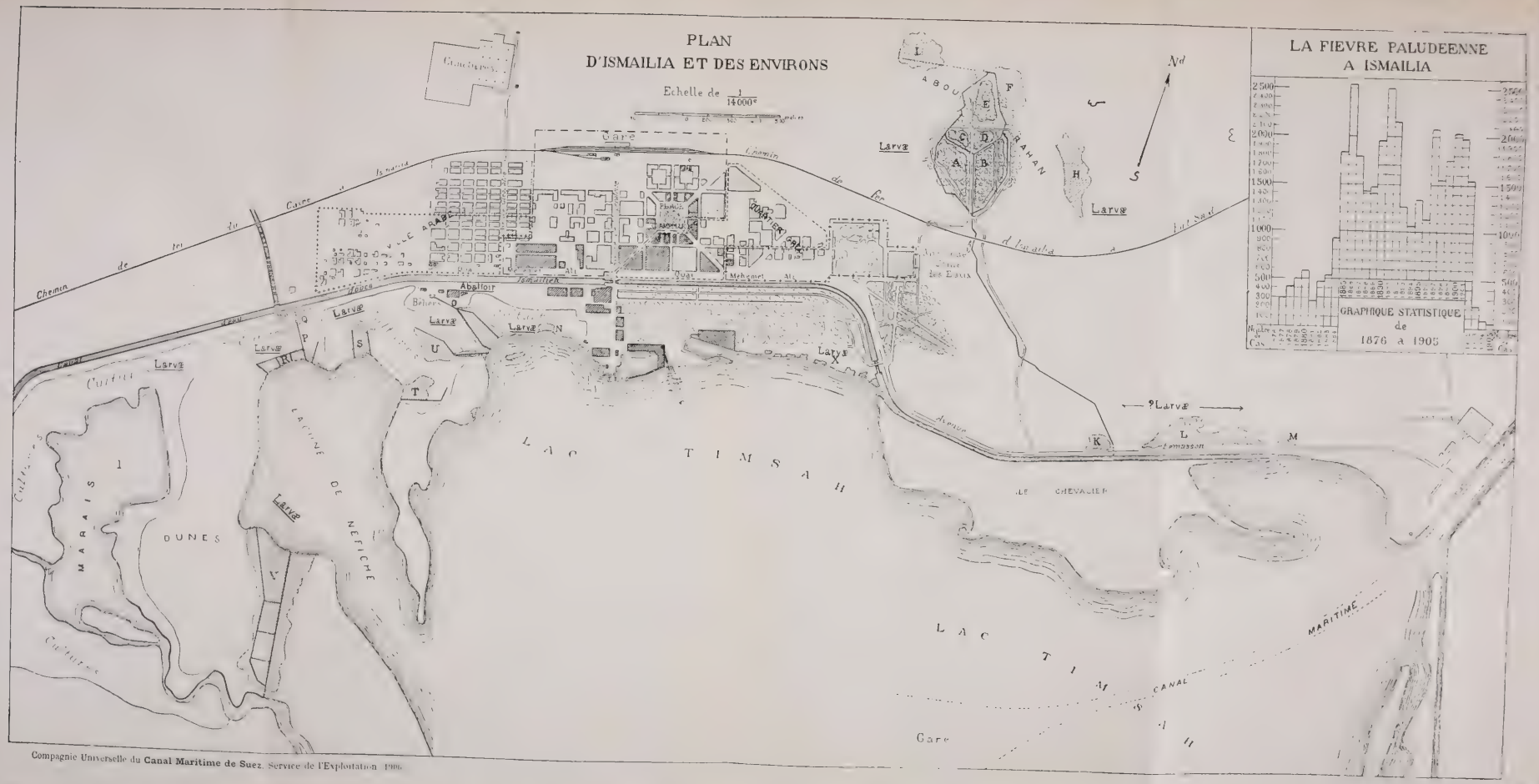
ROUX, J. CHARLES (1901), 'L'Isthme et de Canal de Suez.'

VOISIN BEY (1904), 'Le Canal de Suez, Paris,' 6 vols., 1904-1906.

* *Vide* note antea.

PLAN
D'ISMAILIA ET DES ENVIRONS

Echelle de $\frac{1}{14000^e}$



LA FIEVRE PALUDEENNE A ISMAILIA

Compagnie Universelle du **Canal Maritime de Suez**. Service de l'Exploitation 1906.

Note sur la *Suppression du Paludisme à Ismaïlia.*

ON SOME NEW SPECIES OF AFRICAN MOSQUITOS (CULICIDAE)

BY

R. NEWSTEAD, M.Sc., A.L.S., ETC.,

AND

HENRY F. CARTER,

ASSISTANT ENTOMOLOGIST, LIVERPOOL SCHOOL OF TROPICAL MEDICINE

(*Received for publication* 29 *May*, 1911)

Three of the Anopheline mosquitos described herein formed part of the collections made by our colleague, Dr. Allan Kinghorn, and Mr. R. E. Montgomery during their expedition to Zambesi in 1908, on behalf of the Liverpool School of Tropical Medicine. In addition to these there were also present in the collection examples of *Myzomyia funesta*, *Myzorhynchus mauritianus*, and of the Culicines *Aporoculex punctipes*, *Mansonia major*, *M. uniformis*, and a species of *Chrysoconops*, which may prove to be new.

Neocellia (?) *christyi* was presented to the School by Dr. Christy some years ago. It is a very striking species, but we cannot from the single specimen be quite certain as to whether it is correctly placed in the genus *Neocellia*.

Cellia squamosa var. *arnoldi* has already been referred to by Stephens and Christophers (1908), as *Cellia arnoldi*, and the characteristics of the egg and larva are now given below.

Dr. A. S. Donaldson made an extensive collection of mosquitos while stationed at Broomassie, Ashanti, during the years 1907-1909. It has been our intention to publish a list of his captures, which would add considerably to our knowledge of the mosquitos of this region, but we have thought it desirable to defer this for a future publication. Two new species were found among those he

collected; *Cellia cincta*, described in a previous number of these Annals (1910), and *Reedomyia simulans* described below. We take this opportunity of expressing, on behalf of the School, our best thanks for the valuable material which he was pleased to present to us.

Pyretophorus distinctus, n. sp.

Under pocket lens × 16.

Head.—White in front, yellowish in the centre and darker behind; palpi black, with four whitish bands, the apex white; proboscis black, with the labella pale yellowish brown.

Thorax.—Slaty grey in the middle with a dark median longitudinal line, yellowish-brown laterally; scutellum rather paler in colour than the thorax; pleurae greyish brown.

Abdomen.—Dark brown, with pale golden brown hairs.

Legs.—Femora and tibiae uniformly brown, rather paler beneath; fore legs with distinct but very narrow white apical bands on the metatarsus and first tarsal segments; middle and hind legs with minute pale areas at the articulations.

Wings.—Costa mostly black, with two pale spots extending on to the first longitudinal vein; fringe with pale areas at the apices of the veins.

Microscopical characters.

Head.—Integument dark grey to black, densely covered with upright forked scales, those in front being pure white, those in the median area yellow, and those behind black; projecting from the centre of the anterior portion of the head is a tuft of long white scales and at the margins of the eyes laterally, from four to five dark bristles. *Antennae* grey, the basal segment pale yellow; the second, third and fourth segments with a few white scales. *Clypeus* black. *Palpi* of four segments clothed with very dark brownish grey scales and with an apical band to each segment, the band at the apex of the first being very small and yellowish, the others white. *Proboscis* black, with the labella pale.

Thorax.—With a median longitudinal groove and with two pronounced lateral ridges. Between these ridges the integument is of a slaty grey colour, and on either side of them, dark reddish brown. Anterior area of thorax with a mass of long, thin white

scales, some of which project over the nape, remaining portion clothed with golden narrow curved scales. *Scutellum* denuded, grey in the centre, pale yellowish brown laterally. *Metanotum* dark brown, pleurae rather paler brown. *Halteres* with very pale stems and dark apices.

Abdomen.—Dark brown, with pale golden brown hairs.

Legs.—Brown, femora pale ventrally; first pair of legs with two distinct apical bands on the metatarsus and first tarsal segment, remaining segments of fore legs and also those of the middle and hind legs with pale articulations.

Wings.—With the veins clothed with rather long, thin, black and yellow lanceolate scales; *the costa deep black*, with two pale areas, one apical and the other on the distal half just above the base of the first sub-marginal cell; sub-costa black. First longitudinal vein pale at the base, with five black spots, the apical and two basal ones being the smallest. The second long vein mostly pale, there being two small dark spots immediately before and after the supernumerary cross vein respectively; the upper branch of the cell with a small basal and a large apical spot, the lower branch similar. Third longitudinal vein with a small apical spot and two basal ones almost directly under those on the second long vein. Fourth vein for the greater part pale scaled but with a fairly large, dark area just after the base of the fork; both branches of the cell with two dark patches of scales. Fifth vein with two dark scaled areas, the larger near the centre and extending a considerable distance beyond the junction of the branch, the smaller situated near the apex of the vein, the branch with three spots, one apical and two basal. Sixth vein with three dark patches. Fringe with nine pale areas, situated at the apices of the veins and branches. First cell considerably longer than the second, posterior cross vein about twice its own length distant from the mid vein.

Length.—4·5 mm.

Habitat.—Luapula river, below Chingola's village, N. E. Rhodesia, 17/9/'07. (Dr. A. Kinghorn.)

This Anopheline may easily be distinguished by the dense black costa, which is strikingly characteristic, and also unique among the members of this genus.

Pyretophorus distinctus var. melanocosta, n. var.

This differs from *P. distinctus* in having the *whole of the costa black*, with the exception of the small pale apical spot. There are also other differences, the chief being that the small spot on the apex of the basal segment of the palp is not present; on the first long vein also there are six dark areas as compared with five in *P. distinctus*. The position of the posterior cross vein also differs, it being about its own length distant from the mid cross vein.

Habitat.—Luapula river below Chingola's village, N. E Rhodesia, 17/9/'07. (Dr. A. Kinghorn.)

Cellia pseudosquamosa, n. sp.

Under lens × 16.

Head, white in front, black posteriorly; palpi, dark with three white bands and a small basal spot; on the dorsal surface is a white line running from the apex to the basal spot.

Thorax, dark with two large black ocelli, one on either side of the median line and situated on the anterior portion at a distance of about one-third the length of the thorax; clothed with numerous white scales.

Abdomen, dark brown with dark lateral tufts; last two segments white.

Legs, dark brown, the femora and tibiae mottled, metatarsi and tarsi unbanded.

Wings, dark, very densely scaled; costa with two small and three larger white spots.

Microscopical characters.

Head, dark, very thickly covered with upright forked scales, those at the base black, the rest pure white. A tuft of long white scales projects forwards between the eyes and several dark bristles also extend outwards from the anterior and lateral portions of the head. *Antennae*, dark, the basal segments with a few white scales. *Clypeus*, dark. *Palpi*, with dense brown scales, and white apical bands to the segments, the basal band very small; the upper surface has a thick line of white scales along almost the whole of its length, which gives the two apical segments the appearance of being entirely white. *Proboscis*, black.

Thorax.—Integument dark greenish brown; with two large black ocelli, covered with rather small white spindle shaped scales.

Prothoracic lobes, with outstanding black and white scales. *Scutellum*, dark with white spindle shaped scales. *Pleurae*, paler than thorax with three white longitudinal lines. *Metanotum*, dark. *Halteres*, with pale yellowish stems and dark apices. *Abdomen*, dark brown, densely clothed with long irregular scales, those on the last two segments being white. There are also a few scattered white ones on the 6th, 7th, and also the basal segments; the dark apical lateral tufts are not present on the 7th, 8th and 9th segments. Venter, dark with numerous scattered white scales.

Legs, dark brown, unbanded. Femora and tibiae dark, mottled with white, pale ventrally; the basal half of the metatarsus also shows traces of mottling.

Wings with large black and white lanceolate scales, the greater portion of the wing being dark. Costa black with five white spots, the two smaller basal ones not extending on to the first longitudinal vein, the third being represented on this vein by a few white scales. The first longitudinal has, besides those already mentioned, a small white spot almost under the centre of the second black costal area, and another immediately before the posterior extremity of the third dark costal area; for a short distance at the base the vein is pale. Stem and upper branch of the second vein black, lower branch with two white scaled patches; third longitudinal vein mostly dark with several white scales intermingled with the black ones in the central portion. Fourth vein black; branches of cell with two pale spots and white scales intermingled with the remaining dark portion. Fifth vein with two pale areas on the upper branch and one just before the fork on the lower branch; stem with a white spot towards the apex, base white. Sixth vein with three pale and three dark scaled areas. Wing fringe composed of dark scales but with a few pale scales at the apices of the first longitudinal vein, and the upper branch of the second long vein.

Length.—5·5 mm.

This description was drawn up from a single perfect female, taken by Dr. Allan Kinghorn in North Eastern Rhodesia (Chinyanta's village, Luombwa river), and evidently is closely allied to *Cellia squamosa* Theob. From this, however, it can at once be separated by the unbanded tarsal segments and the somewhat different wing markings.

Cellia squamosa, var. arnoldi. (Newstead and Carter.)

Cellia arnoldi (Stephens and Christophers, 1908).

The only marked difference between typical examples of the imagines of *Cellia squamosa* and the var. *arnoldi* is that the latter has no trace of the white pleural lines; in all other respects the two insects are, so far as we can trace, identical There is, however, a marked difference between the larvae of these insects. In the first place that of *C. squamosa*, according to Hill and Haydon has no branched hair on the antenna, indeed, this structure is apparently absent in many of the known African species of this genus. It would seem therefore that as the var. *arnoldi* has a well developed branched hair, that it may eventually be raised again to specific rank. There are also other differences, especially in the form and situation of the palmate hairs.

Ova (fig. 1), somewhat peculiar in form, the anterior extremity being considerably broader than the posterior. It is reddish brown in colour and about 0·5 mm. in length.

Larva (figs. 2, 3, 4).—*Antenna* with a distinct branched hair on the shaft; terminal hair missing.

Frontal hairs apparently the same as in *Cellia squamosa*, Theob.

Palmate hairs (fig. 4) rudimentary on the first and second abdominal segments, fully developed on the third to the seventh inclusive. In *C. squamosa* the hairs are all well developed on the abdomen, and there is also a rudimentary one on the thorax; the shape and number of the leaflets to each hair also differs from the latter species. In the var. *arnoldi* the filament is very short, and there is a larger number of leaflets.

Neocellia ? christyi, n. sp.

Under pocket lens × 16.

Head.—Dark behind, creamy white in front; palpi dark brown with two white apical bands and a narrower creamy median basal band; proboscis black with the labella pale.

Thorax and scutellum black with creamy scales; metanotum black.

Abdomen dark with distinct pale spots laterally.

Legs brown, femora and tibiae pale beneath with apical banding to the tarsal segments.

Wings large and broad, very clearly spotted costa with five black spots, the second and third the largest, the basal one small and not extending on to the first longitudinal vein. Fringe with eight pale spots.

Microscopical characters.

Head black, clothed with creamy upright forked scales in front, dark ones behind, with a tuft of long irregular pale scales extending distally between the eyes, and with several short golden bristles projecting over the eyes laterally and anteriorly. *Antennae* dark with pale scales on the first few segments. *Palpi* with dark brown scales and with pale apical bands, the posterior pair small and creamy, the distal pair white. *Proboscis* dark.

Thorax.—Integument very dark grey, almost black, with deep cream coloured scales, which approximate more to the narrow curved than to those of the spindle-shaped type; anterior portion with long thin creamy-white scales projecting forward over the nape. *Scutellum* dark, somewhat paler laterally with scales similar to those on the thorax, and with numerous short golden bristles; metanotum black. *Halteres* testaceous with slightly darker apices, clothed with small flat scales of a dull golden brown colour.

Abdomen.—Integument almost black, with white, basal, lateral areas to the middle segments. Covered with long irregular golden brown scales and hairs; the basal segment with a median tuft of long golden bristles. Venter dark with a few white flat scales. *Legs* brown, the femora and tibiae covered with pale scales laterally and ventrally. Tarsi of the first and third pair of legs dark with fairly broad apical creamy bands, the last tarsal segment dark; mid-legs missing.

Wings much broader than is usual in Anopheline mosquitos, the hind margin being markedly arched, and the veins are somewhat thinly scaled. The costa with five dark areas, the third being much the largest, and all, with the exception of the small basal one, spreading on to the first long vein; the first and fourth evenly, the second interrupted by a few white scales in the centre, and the third by a small pale area towards the proximal end. The third and fourth spread evenly on to the sub-costa. Second vein with

a large dark patch at the base, upper branch all black except at the apex, lower with three dark spots; junction of the branches pale scaled. Third vein with three patches of black scales, two basal and one apical. Fourth longitudinal with two large dark areas, each branch with two spots. Fifth vein with three spots almost equidistant, the branch also with three, one at the apex, one immediately in front of the posterior cross-vein and the other at the base of the fork. Sixth vein with two dark areas. Wing fringe with eight pale areas, the first at the apex of the first long vein, the next at the apex of the lower branch of the second long vein, and the others at the apices of the veins.

Length 7 mm.

A single female of this curious *Anopheline* was taken in Uganda by Dr. C. Christy; according to scale structure it appears to belong to the genus *Neocellia*, Theobald, but in its general appearance it is strikingly distinct, and is also widely separated geographically, the members of the latter genus, as defined by Theobald, occurring only in India.

Reedomyia simulans, n. sp.

Under pocket lens × 16.

Head black, with a brilliant white patch in front and a large pale area on each side. *Proboscis* black, with a small indistinct yellowish median band. *Palpi* about the same length as the proboscis with three bands, the two basal ones being little more than spots.

Thorax reddish brown, with two brilliant white shoulder-spots and two smaller ones towards the centre, one on each side of the middle line. Scutellum white; pleurae with five white patches.

Abdomen with basal and lateral spots; apical segment all white.

Legs.—Femora dark brown above, pale ventrally, each with an apical white spot and a small indistinct one a short distance before the apex; tibiae and metatarsi dark brown with apical bands; tarsi of the fore and mid-legs unbanded, the last two joints being pale dusky brown; those of the hind legs with broad apical bands, the last joint being entirely white.

Microscopical characters.

Head (fig. 5) with a triangular patch of silvery white and rather broadly curved scales in front, this patch of scales is

connected by a narrow median line of somewhat narrower curved scales, the latter finally expanding laterally at the base of the head. The remaining portion of the head is clothed with flat scales, the larger sub-median areas being composed of black ones, which, however, gradually merge into lateral basal patches of pure white ones; those scales bordering on the white areas are dark at the base and of a brownish yellow colour towards the apex; these pale areas are followed again with small patches of dark scales. Several upright forked black scales are present over the greater part of the head. Two long dark bristles project between the eyes and also several shorter ones over the lateral margins. *Antennae* testaceous with whorls of long dark hairs. *Palpi* (fig. 6) composed of four segments, slightly longer than the proboscis, and with three small pale bands; the band at the base of the apical segment is the most conspicuous and is composed of white scales, the others at the bases of the second and third are very small and yellowish. *Proboscis* black, with a pale yellow and somewhat indistinct band; labella yellowish.

Thorax reddish brown, covered with narrow curved scales, those on the anterior lateral area forming large white spots; almost immediately under these, towards the centre of the thorax, are two minute patches of similar scales. *Prothoracic lobes* clothed with white narrow curved scales similar to those forming the spots on the thorax; scutellum with white flat scales. *Pleurae* slightly paler in colour than the thorax, with five patches of white flat scales, one of which is situated immediately in front of the root of the wing and another of somewhat dusky scales immediately behind.

Abdomen dark brown with basal dusky white bands expanding laterally into large spots; the apical segment all white. Venter pale dusky brown, the last three segments with basal patches of white scales.

Legs.—Femora dark brown merging into black towards the apex, pale ventrally, and with two white spots, one being apical the other situated on the distal half; tibiae and metatarsi black with broad white apical bands. Tarsi of the fore and middle pairs dark, unbanded, the last two segments brownish yellow; the hind tarsi with broad apical bands on the first, second and third segments, the fourth being all white.

Wings with a white scaled spot at the extreme base of the costa; first fork cell longer and narrower than the second, their bases almost level; stem of the first sub-marginal cell rather more than half the length of the cell, that of the second posterior almost as long as the cell; posterior cross vein rather more than its own length distant from the mid-vein.

Length 3·5 mm.

Habitat.—Broomassie, Ashanti, W. Africa (Dr. A. S. Donaldson).

This pretty little mosquito comes nearer to *R. albopunctata*, Theob. than the other members of this genus, but differs from the latter in the thoracic ornamentation and the leg banding.

LITERATURE

Stephens, J. W. W., and Christophers, S. R. (1908), Practical Study of Malaria, p. 175.

Newstead, R., and Carter, H. F. (1910), Annals of Trop. Med. and Parasit., Vol. IV, No. 3, p. 381.

Hill, E., and Haydon, L. G. (1907), 'A contribution to the study of the characteristics of larvae of species of Anophelina in South Africa.' Annals of Natal Government Museum, Vol. I, Part 2, p. 111.

EXPLANATION OF PLATE XI.

Fig. 1.—Ova of *Cellia squamosa*, *var arnoldi.*

Fig. 2.—Larval head of *Cellia squamosa*, *var. arnoldi.*

Fig. 2a.—Labial plate of same larva enlarged.

Fig. 3.—Lateral comb of larva, *Cellia squamosa*, *var. arnoldi.*

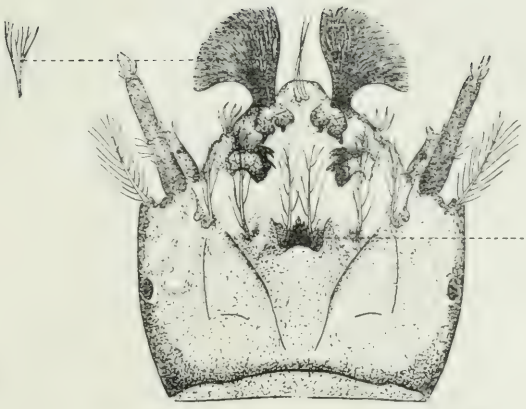
Fig. 4.—Palmate hair from third abdominal segment of larva, *Cellia squamosa*, *var. arnoldi.*

Fig. 5.—Cephalic scaling of *Reedomyia simulans*, n. sp.

Fig. 6.—Head and palpi of *Reedomyia simulans*, n. sp. ♂.



1



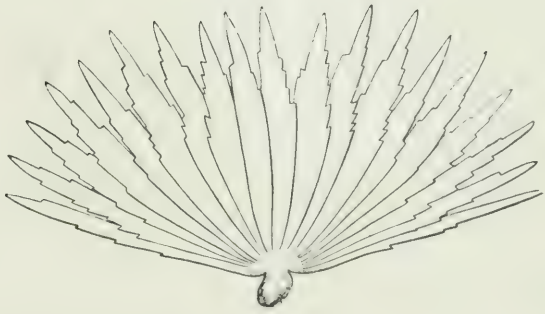
2



2a



3



4



5



6

H. F. Carter, del.

THE DIAGNOSIS AND DISTRIBUTION OF HUMAN TRYPANOSOMIASIS IN THE COLONY AND PROTECTORATE OF THE GAMBIA

First Report of the Expedition of the Liverpool School of Tropical Medicine to the Gambia, 1911.

BY

JOHN L. TODD, M.D.,
ASSOCIATE PROFESSOR OF PARASITOLOGY, MCGILL UNIVERSITY, MONTREAL

S. B. WOLBACH, M.D.*
ASSISTANT PROFESSOR OF BACTERIOLOGY, HARVARD UNIVERSITY MEDICAL SCHOOL, BOSTON

(*Received for publication* 12 *June,* 1911)

CONTENTS

1. PREFACE ... 246
2. INTRODUCTION ... 248
3. PROCEDURE ... 255
4. TECHNIQUE ... 257
   (*a*) Fresh cover-slip preparations ... 257
   (*b*) Thin blood smears ... 257
   (*c*) Thick blood films ... 257
   (*d*) Centrifugalisation of the blood ... 258
   (*e*) Auto-agglutination ... 258
   (*f*) Gland palpation ... 258
   (*g*) Gland puncture ... 259
5. FINDINGS.
   (*a*) Fresh cover-slip preparations ... 259
   (*b*) Thin blood smears ... 260
   (*c*) Thick blood films ... 260
   (*d*) Centrifugalisation of the blood ... 260
   (*e*) Auto-agglutination ... 260
   (*f*) Gland palpation and puncture ... 261
   (*g*) Pulse and temperature ... 273
6. THE METHODS OF DIAGNOSING TRYPANOSOMIASIS DISCUSSED AND COMPARED ... 274
7. THE PRACTICAL APPLICATION OF GLAND PALPATION AND PUNCTURE ... 280
8. NATIVE TOLERANCE OF TRYPANOSOMIASIS ... 282
9. RECOMMENDATIONS ... 283
10. CONCLUSIONS ... 286

*Sheldon Fellow in Tropical Medicine, Harvard University.

I. PREFACE

The main objects of an expedition sent to the Colony of the Gambia, in 1911, by the Liverpool School of Tropical Medicine, were to make additional observations on the efficiency of gland puncture in the diagnosis of human trypanosomiasis, and to determine the incidence of that disease in the territory visited by the expedition. The present paper reports on this part of our work; observations on other points will be reported on in later papers. That a few cases of human trypanosomiasis may usually be found in the Gambia is shown by the discovery there, in 1901-02, of *Trypanosoma gambiense* in two Europeans and in six natives[1]. Since then the parasite has been found in one other European and in [4] several natives. In a report made for the Under Secretary of State, in 1910, Dr. Hopkinson states that there are about six cases of trypanosomiasis among the 1,500 new cases whom he sees yearly; he suggests that about 1 per cent. of the patients coming to the hospital at Bathurst are cases of trypanosomiasis[4]. The figures published in the report of the Senior Medical Officer, Dr. Hood, for 1909, would make it appear that the number of cases is rather less than this; for there were no cases of trypanosomiasis recorded among almost 8,000 patients treated during that year at the Bathurst Hospital, and only one among 1,117 treated at the McCarthy Island Hospital. In 1908, however, there was one death from trypanosomiasis among 593 patients admitted to the Bathurst Hospital.

All of these observations prove that human trypanosomiasis has been endemic in the Gambia for some years; the Gambia, consequently, offered an excellent field for testing the efficiency of gland palpation and puncture in the diagnosis of human trypanosomiasis. The Gambia furnished an exceptionally good opportunity for a test, since it has been suggested that gland palpation is most likely to prove untrustworthy in those localities where human trypanosomiasis exists in an endemic, rather than in an epidemic form. It has often been suggested that palpation, controlled by gland puncture, could not be usefully employed in detecting cases of trypanosomiasis, because many natives who live in areas where sleeping sickness occurs sporadically, have enlarged

glands without obvious cause, and that all of them could not possibly be infected with trypanosomes. Indeed, when human trypanosomiasis was first described in the Gambia[1], it was considered inadvisable to lay much stress on the diagnostic importance of the enlarged lymphatic glands existing in the cases in whom trypanosomes were found, since no trypanosomes were found in the blood of other, apparently healthy, natives whose glands were also enlarged. Eventually, experience in the Congo Free State led to the publication of reports in which it was concluded that, in areas where human trypanosomiasis exists, all negroes with enlarged glands must be considered to be cases of trypanosomiasis until the contrary is proved, and, although it was recognised that all cases of the disease are not detected by gland palpation and puncture, it was urged that this fact should be used as a basis for measures designed to control the spread of sleeping sickness. Many papers have since appeared on the same subject. Some authors conclude that gland palpation and puncture form an efficient diagnostic method, others do not. Those who decry it have usually expected too much from it. Most of the papers which had appeared up to August, 1908, have been reviewed and discussed[3] by one of us. Those which have appeared since then have been abstracted in the Bulletins of the Sleeping Sickness Bureau. These Bulletins also contain full considerations of the literature dealing with the measures employed in the diagnosis of human trypanosomiasis, and the Director of the Sleeping Sickness Bureau has published reviews of them[5, 12]. Broden and Rodhain[6], Thiroux and d'Anfreville[7], the German Sleeping Sickness Commission in Togoland[8], the Sleeping Sickness Commission in French Congo[9], the German Sleeping Sickness Commission in East Africa[10], Kinghorn and Montgomery[11], Davey, Stannus, Park and Barclay[13], Horn[14], Kinghorn[15, 16, 21], Sanderson[18], May[19], Drew[20], have all written papers on this subject. All these authors have employed gland palpation and puncture, and have found them of value; there is, however, considerable variation in the opinions they express concerning the exactness of the results obtained by these methods.

References are given to the abstracts in the Bulletins rather than to the original papers, because the former are much more easily

obtained by most persons. It would be unprofitable to enter upon a second consideration of these papers; especially since one would necessarily traverse, almost exactly, the ground covered by a previous review[3]. More recently Kinghorn and Montgomery have given an excellent review of the subject[11].

The figures obtained by our work in the Gambia speak for themselves; the results there entirely coincide with those obtained in the Congo. Consequently, the conclusions reached in the Congo are left unaltered. It seems strange that our results should differ so widely from those of other authors. Koch stated in East Africa that '50 per cent. of trypanosome carriers could be detected by a single examination of the blood'; the statement would have been impossible if the carriers examined had been the early cases occurring in the Gambia and in the Congo Free State.

Thanks are due by us for favours and assistance received during this expedition from the Elder Dempster Steamship Company, from the Governor, the officials and merchants in the Colony of the Gambia, and from M. Legrand and M. Lanzerac in French territory.

II. INTRODUCTION

Because human trypanosomiasis is endemic in the Gambia, that territory offered an excellent opportunity for testing the efficiency of gland palpation and puncture in the diagnosis of that disease. The expedition sent out for the purpose determined to examine as many natives as possible in all parts of the colony, in order to avoid any errors which might be produced by local causes.

The expedition reached Bathurst, the capital of the Colony of the Gambia, on the 4th of February, 1911. Five days were spent in making the necessary arrangements, and work was commenced on the 10th of February. Ninety days were spent in travelling through the Protectorate and five in examining Bathurst and its neighbourhood. In all, the expedition spent one hundred days in the Gambia. During that time it travelled about 550 miles, and it palpated the necks of 12,298 natives drawn from ninety-five towns and villages. Trypanosomes were found in seventy-nine persons. If to these be added twenty-one persons with much enlarged glands, whom it was impossible to puncture and who

were almost certainly infected, a total of one hundred is obtained; consequently, at least, 0·8 per cent. of the whole population of the Gambia are probably infected with trypanosomes. From the observations made by previous expeditions to the Gambia, and from the reports made by resident medical officers[4] it was already known that *Glossina palpalis* was very common everywhere along the Gambia and its tributaries, and that *Glossina morsitans* also occurred there. During this expedition *Glossina palpalis* was seen in varying numbers, wherever the neighbourhood of the river, or of a stream, was remained in for any length of time. Several areas, on both sides of the river were passed through in which swarms of *Glossina morsitans* occurred, and it was seen in very many places, far from any water, in smaller numbers. No reason was observed for the irregularity in their distribution. Tabanids and sand-flies were very common.

On the accompanying map the towns visited are underlined, and the route followed by the expedition is indicated by an unbroken line; so far as it was possible, examples of every type of country, included in the 5,000 square miles of the Colony and of the Protectorate of the Gambia, were visited.

The expedition was undertaken during the dry season because it is almost impossible, for Europeans at least, to travel in the Gambia during the rains. The dry season there lasts from about November to June.

During the height of the dry season there is very little water in the country. The swamps are dry and the river becomes little more than an arm of the sea in which fresh water lies; at this time of the year the tides are felt at Fatta Tenda, about 240 miles inland by the river from Bathurst. There is, consequently, very little fall to the river, and the country through which it runs is very flat. During the rains the river is swollen so that it sometimes passes the banks and its current becomes so rapid that sailing boats require weeks to make journeys which can be made in days during the dry season. At this time of the year the swamps and creeks are all flooded so that it becomes almost impossible to travel by any of the roads running near the river.

At its mouth, and for some eighty miles up stream, the river and the creeks tributary to it are bordered by dense fringes of

mangrove trees. The fringe of mangroves varies in width from ten to fifty or more yards. Beyond the mangroves there are often extensive grass-covered, swampy plains; such plains are very characteristic of the Gambian Protectorate, and they occur on both sides of the river all the way from the sea to the end of British territory, some 200 miles inland. From the swamps the land rises gradually to the level plain, of which the greater part of British territory consists. This plain is composed of sandy soil and, where it is uncultivated, is usually covered by forest composed of scrubby trees and bamboo. Near water-courses, or on low-lying ground, there are a few heavily forested areas. The plain varies considerably in width, but practically all of it is included in British territory, which is merely a strip of land, ten kilometres in width, on both sides of the winding river. It is evident that the Gambia has not always occupied its present bed, and that the level country on either side of the river is part of the wide valley which it has eroded. The valley is limited more or less abruptly by higher ground. Sometimes it is limited by high escarpments of red, volcanic iron-stone or by cliffs going down many feet to the river; sometimes the rise is gradual to a plateau only a few feet above the river, which is covered by scrubby forest and interrupted by out-croppings of the constantly recurring, red, volcanic rock.

A few Niuminkas living at Bathurst are fishermen by profession. Some of the Mandingoes and Jolloffs living along the sea-coast or in villages situated near the banks of the river, in the lower part of its course, own canoes and often catch fish. With these exceptions the whole of the population of the Gambia is agricultural or pastoral, and no tribe gains any considerable part of its food from the river. Almost every village, however, looks forward to scooping, with hand-nets, a few fish from the ponds left in the dried-up swamps at the end of the dry season.

The population of the Gambia consists mainly of Mandingoes, Jolloffs and Jolahs; these tribes are mentioned in the order of their importance. There is also a considerable number of Foulahs. The customs of Mandingoes, Jolloffs and Jolahs are very similar; they are all agricultural peoples who build their villages near their fields, and grow ground-nuts, millet of several varieties, rice, beans, Indian corn, gourds, pumpkins, and medicinal herbs of various

sorts, as well as cotton. The chief difference between them is that the Jolahs are more primitive than the other tribes. Unlike them, they are not Mahomedans; they drink palm wine and live in primitive hamlets, not in villages. They are very independent and much more difficult to control than their more civilised neighbours; if it were advisable to do so it might be difficult to persuade them to adopt measures designed to prevent the extension of sleeping sickness among them. With the exception of a few insignificant Mandingoe villages almost all of the native towns in the Gambia are built at some distance—half a mile or more—from the banks of the river. Indeed, one Mandingoe chief said that his people know that 'it is not healthy to build a town near too much water.'

The Foulahs are pastoral people. They are most numerous far up the river; but they occur throughout the Colony. They move, with their cattle, from grazing-ground to grazing-ground. In the wet season they leave the river for the interior, where their cattle are not exposed to fly-bites; in the dry season they return to the river for the sake of the grass and water in the swamps along its banks. Consequently, the Foulahs build no towns but, at the most, only temporary collections of huts.

All the tribes in the Gambia are very prosperous. Their cattle have increased enormously in number and their land is fertile. They raise good crops of millet and rice for their own use, and large amounts of ground-nuts which are sold to traders for export to Europe. With the money obtained from the sale of ground-nuts the natives are able to buy all the European articles, such as cloth and powder, which they require. Because of the favourable conditions for obtaining money by the sale of ground-nuts, large numbers of young men yearly come to British territory in order to make farms and raise crops of ground-nuts. They come from French territory, from all directions; some of them come from places distant eight, and even more, weeks' travel.

Eleven cases of clinical 'sleeping sickness' were seen during the expedition. Although the disease has existed in the Gambia for some years, and although single cases have been seen in every part of the colony, it has never become epidemic; neither do cases ever seem to have been very common. In 1902[2] the blood of 1,043 natives was examined by cover-slip preparations; six of them,

0·5 per cent., were found to be infected with trypanosomes. During this expedition the blood of 362 persons, selected by gland palpation, was examined in exactly the same way; trypanosomes were found in seven, 1·9 per cent. After examining three cover-slip preparations the parasites were found in the blood of an eighth case, in whose gland-juice they had been seen previously. From these figures it seems possible that human trypanosomiasis in the Gambia may be tending to increase. It is interesting that three cases were found in Lammin among 100 persons examined, in 1902; in 1911 three cases were found there among thirty-five persons whose blood was examined.

The natives of all the tribes know the disease well (the Mandingoes call it *Kanta bero*,* the Jolloffs, *Nelouan*, and the Foulahs, *Doïngol* or *Danu*). Nevertheless, answers to our questions concerning the presence of sleeping sickness were sometimes given which seemed to be almost wilful in their strangeness. For example, the chief at Essau, where 5·4 per cent. of the population had trypanosomiasis, professed to be able to remember only one case of sleeping sickness among the people of his village.

Every headman was questioned; but none gave any hint of a tradition that sleeping sickness had ever been more prevalent than it is at present, and none knew when the disease first came to the Gambia, though they all agreed that it had been in the country for two or three generations. Natives told Dr. Hopkinson that, formerly, no towns were built near Nianija Bolon (creek) on the north bank because persons living there were in danger of catching sleeping sickness. It is probable that the situation of the native towns, among fields at some distance from the river, and the agricultural habits of the natives—which make it unnecessary for the men to go frequently to the river—have had some effect in preventing the spread of the disease.

M. Legrand, the Administrator of the French Territory to the South of the Gambia, wrote that he has been travelling through the district of Fulladu for two years, and that all the natives living in that district are well acquainted with sleeping sickness. He, with Dr. Dufougére, estimated that about 0·15 per cent. of the natives

* This term really means 'neck stones' and refers to the enlarged glands; *Sina jankers* refers directly to the disease.

there were infected with trypanosomiasis. Most of the cases were young men who had gone to British territory along the Gambia to farm during the rainy season; but tsetses do exist in Fulladu about the marshes, so that cases of trypanosomiasis do occur there among natives who have never been to the Gambia. M. Lanzarec, the French resident in the territory to the North of the Gambia, had also noticed that sleeping sickness occurred most frequently among those natives who had farmed in British territory. It is certain that the men who cultivate millet and ground-nuts in the Gambia do run some danger of being bitten by tsetse-flies. Natives often stated that they were bitten while they were at work by flies, which they called *Solo-fing jolo* or *Kongjolo*. Both *Glossina palpalis* and *G. morsitans* are probably included under these names; we saw no other small species of tsetse-flies in the Gambia. It was frequently said that the flies were most numerous in the wet season and that —this we saw—*Glossina palpalis* often followed persons who had come from the water-side into towns situated half a mile or more from the river bank. It is, however, the women who are most exposed to tsetse-flies. They alone cultivate rice. The rice-fields are always placed in the swamps, and they often lie within a hundred yards or so of the river, consequently, those who work in them, as the natives freely admit, must be frequently bitten by tsetses.

A French trader, who passed the rainy season of 1909 at Jamekunda, near Sallikeni, said that this town has many rice-fields which are situated near an arm of the river. As is usual, they are worked by the women, with the result that many women have died of sleeping sickness, and one man lost five wives from that disease in two years.

Young children are usually carried wrapped in a shawl on their mothers' backs. They are consequently little exposed to infection. Boys are sent on errands, and run about everywhere; while girls help their mothers in household work, in drawing water and in farming; so both boys and girls are exposed to the bites of flies; the boys are, perhaps, more exposed than the girls. As has been pointed out, the women are much more exposed than are the men, because they cultivate rice. In maturity and in old age both men and women, provided they have children or slaves to maintain them, do little work afield, and remain very largely within the villages. A consideration of the usual occupations of the natives in the Gambia would lead to the conclusion that in childhood males

are more likely to be bitten by tsetse-flies than are females; but that in adult life, where rice is grown, females are more likely to be bitten than men and, moreover, that females are likely to be bitten more often than any other class of the population. It would consequently seem as though the women were more liable to become infected by trypanosomes than are men. An inspection of Table III is interesting in this connection. It may be noted here that because of their habits the pastoral Foulahs are little exposed to infection; few of them have much enlarged glands and no case of trypanosomiasis was found among them.

In the Gambia, as in many other places along the West Coast of Africa, the natives of all conditions and tribes realise that the occurrence of enlarged glands is one of the earliest signs of 'sleeping sickness.' Some of them, at least, also recognise that change of character, unstable emotions—easily excited tears or laughter—and irritability are often early signs. Dr. Hopkinson has been told by natives that an early sign is delay of the eyelids in following the eyes when an affected person looks down. Many natives realise that frenzy and mania are often late symptoms of the disease which may exist before somnolence appears. One headman had noticed that persons who scratched much often had sleeping sickness.

Many tribes along the West Coast of Africa practice gland excision as a preventative of sleeping sickness; the Jolloffs, Mandingoes and, apparently to a less extent, the Jolahs and Foulahs all do so. Sometimes glands are undoubtedly removed with a knife and a bit of wire. Often none can have been reached[1] through the incision which is very frequently made high up, on the ramus of the jaw, in a situation from which it would be almost impossible to remove a gland; in these instances it seems almost certain that no glands were removed. In other cases incisions have been made in favourable places beneath the jaw, in the anterior or posterior triangles of the neck and in the axillae; in these instances it seems very probable that glands were excised. There are some men who profess to remove glands by plasters, which 'draw' them out. Something of the same sort is done by natives in Nigeria and on the Ivory Coast.

The natives, even those who are most educated, believe in the

advisability of gland excision, and believe, more or less firmly, that the removal of glands will prevent the development of sleeping sickness. They usually say, however, that excision is only of value in the early stages, and that it is useless to remove glands when the sickness has 'gone into the body.'

Very many of the natives living in the Gambia have had glands removed. The fact that 150 out of 220 consecutive persons chosen for gland puncture had had glands excised will give some idea of the extent of the practice. In some districts excision is more general than in others; the Jolahs seem to practise it least of all. Usually, the operation is done but once on each individual; not infrequently, persons are met with who have, as they say, had their 'bumps pulled' on several occasions. Some of these have trypanosomiasis.

One woman, aged 24, in whom trypanosomes were found, had had glands removed when she was about 13 years old. Since then, on three occasions, glands were said to have been removed from the sub-maxillary, posterior cervical and axillary groups.

One case of clinical trypanosomiasis had had glands excised on five occasions during as many years.

Several persons in whom trypanosomes were not found had had their 'bumps pulled' three times; usually the operation is only done once, in childhood. The natives all know that people above middle age rarely have enlarged glands and, consequently, that they rarely have sleeping sickness (see Table I).

III. PROCEDURE

It is estimated that there are over 200,000 negroes in the Gambia. In order that those whom we examined might represent a fair sample of the whole population, persons were examined in every part of the Colony and Protectorate. As many persons as was possible were examined in each village visited. The natives were not called together for us to see them, but we went from house to house and entered the huts in order to make certain that cases were not being concealed. As the posterior cervical glands of each native were palpated, the age, sex, and degree or absence of enlargement of the glands was dictated to a clerk who noted them; these notes were kept for a series of 9,069 persons. Notes of only those with puncturable glands were made for the remainder of the 12,298 persons palpated.

So soon as palpation of a village was finished, all of those who had puncturable glands were told to go to the camp of the expedition to be examined. One of the objects of the expedition was to compare the efficiency of the various methods of diagnosing trypanosomiasis; so, in a series of 283 persons, observations on the following points were made for each native examined: personal history, pulse, temperature, size of spleen, whether the glands had been excised, presence of enlargement in all the groups of superficial lymphatic glands, the presence of scurf, tartar, skin disease, or of any cause which might produce glandular enlargement, and the presence of trypanosomes in gland juice or blood. The blood was searched for trypanosomes by the examination of fresh cover-slip preparations, of thick films and of smears. At first it was intended to also centrifugalise the blood by either our own method or by that first employed by those who worked in Uganda[5]. Both of these methods are tedious, and it soon became evident that it would be necessary to abandon all hope of using them if a serious number of gland punctures was to be done in the time at our disposal. In the remainder of the 350 persons whose glands were punctured, the full examination was made on only those in whom trypanosomes were found; for the others the results of the examination of blood and gland juice alone were recorded.

The names and descriptions of all those who were punctured have been given to the Senior Medical Officer and to the Commissioner of the district in which they were seen. It is hoped to keep track of them, and in this way to gain information on at least two very interesting points, namely: 'Do all untreated cases of human trypanosomiasis in natives end fatally?' and 'Are any of the persons with moderately enlarged glands, and in whom no trypanosomes could be found, really cases of trypanosomiasis?' With the exception of the young men, natives in the Gambia travel but little, because they are prosperous and contented. They are well under control and they are usually very amenable to European rule. It is, consequently, probable that it will be possible to keep track of these persons. It is very important that they should not be lost sight of; the probability that they could, and would, be kept under observation was one of the reasons which determined the sending of this expedition to the Colony of the Gambia.

IV. TECHNIQUE

In order to establish a just basis for comparing the various methods of diagnosing trypanosomiasis, a definite routine of examination was established.

All the observations on each case used in making our comparison were made at the same time. The blood preparations were made with blood drawn from a single finger. The gland punctured was usually the most convenient one in either of the posterior cervical groups. If glands from any other group were punctured the fact has been noted. It has been strongly insisted that only perfect specimens of gland juice should be examined, for a negative examination of an imperfect preparation of gland juice has absolutely no significance[2]. Consequently, only examinations of good preparations of gland juice and of blood have been recorded. As a rule only one preparation was made by each method of examination, if more were examined the fact has been noted. A microscope used in searching for trypanosomes should always be fitted with a mechanical stage so that it becomes possible to make certain of missing no part of the preparation examined.

A. *Fresh cover-slip preparations.*

The simplest method of finding trypanosomes in a patient is to place a drop of his blood between a slide and a cover-slip and to search in it for the living parasites with a microscope. The trypanosomes are detected by their movement, so the preparation must not be too thick lest the movements of the parasites should be obscured by the blood cells. As usual, our fresh preparations were made with cover-slips, three-quarters of an inch square, and they were examined with a Zeiss, D. objective and a No. 4* eye-piece.

B. *Thin blood smears.*

In making a thin smear a drop of blood, half as large again as that used in making a cover-slip preparation, is placed at one end of a slide. Then, with a needle, placed transversely across the drop, the blood is smeared along the slide in a thin layer. Our smears were allowed to dry and they were then fixed in absolute alcohol and stained by Giemsa's method.

C. *Thick blood films.*

Four or five drops of blood as large as those used in making thin smears, are placed on a glass slide over a circular area about

one centimetre in diameter. The slides are then usually fixed by heat, de-haemoglobinised in distilled water and stained by some modification of Romanowsky's method. The value of the method depends upon the well-known manoeuvre of removing the haemoglobin; haemoglobin stains densely so that, were it present, it would be impossible to see parasites lying among the red blood cells.

D. *Centrifugalisation of the blood.*

Only a few specimens of blood were centrifugalised. We are quite aware of the advantages offered by the method, but the length of time required for employing it, as well as the large amount of blood, 10 c.cm., required for the most usually employed method of centrifugalising, makes it almost impossible for it to be used in the routine examination of a large number of persons. We regret that those in whom trypanosomes were found by other methods were not examined by this one; but lack of time and the fear of frightening natives by taking the necessary blood from them prevented us from doing so.

E. *Auto-agglutination of the red blood cells.*

It has been noted frequently, in fresh cover-slip preparations, that the red blood cells of persons suffering from trypanosomiasis very often run together to form shapeless masses. The term of auto-agglutination has been applied to this phenomenon.

F. *Gland palpation.*

The classification employed for grouping the persons palpated according to the absence of glandular enlargement or according to the degree of it, if it were present, was that proposed in the Congo[2]. Those with enlarged posterior cervical glands are grouped in the following way, according to the degree of enlargement present. As '+' are classified persons with posterior triangles which contain (*a*) one gland which is estimated to measure at least 1·5 by 0·75 cm., or (*b*) three or more smaller glands, the largest measuring perhaps 1 by 0·5 cm. As '+ —' are classified enlargements, less than this, but greater than those classified as '+ — —.' As '+ — —' are classified groups containing only one or two glands measuring 0·5 by 0·25 cm., or (*b*) many tiny shot-like glands which are only just palpable.

G. *Gland puncture.*

No change was made in the technique developed in the Congo Free State[2]. Ordinary hypodermic syringes were used. The gland was fixed with the left hand while the needle of the syringe was passed into it, with a sharp thrust, by the right. The plunger was withdrawn by an assistant while the point of the needle was gently moved about within the gland. The plunger was released and the syringe withdrawn. The tiny droplet of fluid, which was usually all that was obtained, was then blown out by a single thrust of the plunger, and examined immediately in the same way as a fresh preparation of blood was examined. The necessity of avoiding the dangers mentioned in former papers[2] was more than ever evident. The preparations must be thin, and care must be taken to use clean slides and cover-slips and to see that the morsel of skin punched out by the needle is not contained in the specimen. If the preparation contains water from the syringe, or air bubbles, it is not a good one, and the chance of finding living trypanosomes in it is lessened. Trypanosomes are also less likely to be seen in pus-like preparations, in which the cells are dull, than in preparations filled with clear, brightly refractive cells. Those who attempt to examine a person suspected of trypanosomiasis by gland puncture must not record a negative examination until they have examined, in perfect preparations, all of the material obtained by the successful aspiration of a gland. Although there is usually very little blood in a preparation of gland juice, the presence of even a considerable number of red cells seems to make no difference to the likelihood of trypanosomes being found.

V. FINDINGS

A. *Fresh cover-slip preparations.*

The blood of 362 persons was examined by cover-slip preparations; trypanosomes were found in eight of them. A series of 340 persons were examined by gland puncture and by cover-slip preparation; gland puncture detected sixty-six cases of trypanosomiasis, including all of six instances in which simultaneous examination of cover-slip preparations was successful in finding trypanosomes. It was necessary to examine three cover-slip

preparations from one of these cases before the parasites, already seen in gland juice, could be found in the blood.

B. *Thin blood smears.*

Thin blood smears were examined from 316 persons. Trypanosomes were found in ten of them; the parasites had also been seen in three of these cases in cover-slip preparations made at the same time as the smears. They were also found by the examination of cover-slip preparations in five persons in whom the examination of thin smears had failed to find them. Gland puncture found forty-eight cases of trypanosomiasis among these 316 persons.

C. *Thick blood films.*

Thick blood films, prepared in the manner described, were examined from 265 persons. Trypanosomes were found in only eight of them by this method, while they were found in forty-seven by gland puncture, in ten by the examination of thin smears, and in five by the examination of cover-slip preparations. All the thick films in infected cases were examined during half an hour at least. One film of blood in which trypanosomes had been found by thin smears was examined without result for three-quarters of an hour and another one was examined in the same way for an hour. Three thin smears were examined without finding trypanosomes in a case in whom the parasites had been found by the thick film method.

D. *Centrifugalisation of the blood.*

The blood of only three persons was centrifugalised. Ten c.cm. of blood was drawn from a vein in each case and centrifugalised three times; over an hour and a half was spent in the preparing and examining of from two to four cover-slip preparations from each person. Trypanosomes were found in none of them, although they had been previously found by gland puncture in two of them.

E. *Auto-agglutination.*

When it was first described, it was decided that auto-agglutination was not always present in trypanosomiasis, but that it often did occur in persons and in animals who were suffering from that disease, and it was concluded that though auto-agglutination

had no diagnostic value it frequently encouraged a successful search for trypanosomes in persons in whom they were not at first found.

A good deal of attention has been given to auto-agglutination recently[24]. For this reason the cover-slip preparations made from 350 persons, whose glands were punctured, were examined for auto-agglutination. These persons have been classified, in Table I, according as trypanosomes were found in them or not. They are further divided into four classes according to the absence of auto-agglutination, or according to the amount of it which was present. An inspection of the table shows that well-marked auto-agglutination may be present in persons who are not infected with trypanosomes, and also that some degree of auto-agglutination is very constantly present in persons who are infected with trypanosomiasis, although it may be absent altogether from some of them. It is worth noting that trypanosomes were probably present in two of those persons in whom the parasites were not seen, although auto-agglutination was well marked; most of our cases were only examined once and the presence of a slight temperature with enlarged glands and auto-agglutination makes it probable that trypanosomes would have been found in these two cases had the search for the parasites been persevered in.

Table I

	Marked agglutination	Slight agglutination	Very slight agglutination	No agglutination
Trypanosomes not present ...	11	37	59	176
Trypanosomes present	26	15	14	12

F. *Gland palpation and puncture.*

The classification of persons with enlarged cervical glands which was proposed in the Congo Free State has been retained. This classification has been adhered to because it seems to be a reasonable one and a useful one. It is reasonable because nothing can be clearer and less 'mysterious' than reference to actual measurements, and because it can be appreciated by every doctor; many physicians

have never seen ' familiar objects,' such as pigeon's eggs, filberts and pfennig pieces, but every medical man should have a very accurate knowledge of the length of one centimetre. It would be misleading to divide glands into puncturable and unpuncturable, because after a little practice, anyone can draw gland juice from glands which he would, at first, have thought unpuncturable. It is a useful classification because the results of our examinations show every negro with ' + ' glands, without some evident cause, is, almost invariably, a case of trypanosomiasis. This is especially so if the enlarged glands have the thickly fluctuating consistency to which many observers have alluded (Grey and Tulloch[5], page 61). As a rule, only a few of those classified as ' + — ' have trypanosomes, while almost none of those classed as ' + — — ' have been found to be infected. These points are illustrated by the following table of those with enlarged glands seen among the 12,298 persons who were palpated in the Gambia.

Table II

Degree of glandular enlargement	+	+ —	+ — —	Total
Seen	56	136	2102+	2294+
Punctured	36	63	233	332
Trypanosomes found	36	28	4	68

It will be noticed that trypanosomes were found in the gland juice of all of the thirty-six ' + ' cases. The parasites were also found in twenty-eight out of sixty-three ' + — ' cases; they were also seen in four out of 233 ' + — — ' cases. The number of cases classed as ' + — ' and ' + — — ' in whom trypanosomes were found is, proportionally, much larger than it was in the Congo[2].

It will be noticed from Tables II and III that there were a considerable number of infected persons among those who were classified as ' + — ' and ' + — — '. This is to be explained, in part at least, by our having classified those in whom there was a difference between the degree of enlargement present in the posterior triangles of the neck according to the triangle which showed the lesser enlargement. Consequently, of the four positive cases who had

' + — —' glands on one side, two had ' + ' glands on the other side of the neck, and two had ' + —' glands.

The posterior cervical glands were taken as an index of the general enlargement of lymphatic glands which occurs in human trypanosomiasis, just as in the Congo, because they are the groups least exposed to the usual casual causes which produce enlarged glands.

In order to ascertain whether the enlargement of lymphatic gland groups was general in persons infected with trypanosomes, and in order to ascertain whether enlarged glands frequently occurred from other causes than trypanosomiasis in the Gambia, all of the gland groups of 312 persons were palpated. Dr. Hopkinson states[4] that the Jolloffs have enlarged glands more frequently than members of any other tribe. We did not notice that difference, but it was apparent that the Foulahs, and the few Jolahs whom we saw, had fewer persons with enlarged neck glands that had the Mandingoes and Jolloffs who lived near them.

As a rule, all early cases of trypanosomiasis had a considerable degree of general glandular enlargement, but there were six early cases who had one or more gland groups, usually the axillary, epitrochlear or sub-maxillary, not at all or only slightly enlarged. A little watching, however, would probably have discovered signs in at least one of these cases, who had a temperature of 100° F., which would have caused it to be called a well advanced case; it is well known that the size of the lymphatic glands diminishes in advanced trypanosomiasis, and that the value of gland palpation and gland puncture is least in just those cases which can be recognised more easily by clinical, rather than by laboratory methods.[2]

An inspection of the results of the palpations (Table III) shows that children, boys especially, are most likely to have enlarged glands; this is probably because of their lack of personal cleanliness.

Boys often have more or less generalised skin disease, usually 'craw-craw,' they very frequently have a good deal of scurf, and almost always a certain amount of tartar. The frequency of foul mouths and of a high grade of pyorrhoea is very great among the Gambian natives of all ages and classes. All these things often

caused enlarged glands; the slightly enlarged posterior cervical glands of a large number of boys were certainly due to scurf; while enlarged sub-maxillary glands, in persons who had no other groups enlarged, were usually due to an infected mouth. Lice, and cuts or scratches on various parts of the body were also frequent causes of enlarged glands. A considerable degree of enlargement of the groin glands was almost universal among men.

Four or five of the persons in whom trypanosomes were found had evident reasons for their enlarged glands; so the presence of an adequate explanation, such as a dirty, crust-covered head, for enlarged cervical glands by no means excuses their not being punctured. This is especially so since lack of personal care is very usual in established trypanosomiasis.

It is worth repeating—'until the contrary is proved every native with enlarged glands must be suspected of trypanosomiasis.'

Table III shows the incidence of enlarged glands and of trypanosomiasis among 9,069 natives who have been classified according to their sex and age in order to ascertain whether any class in the Gambia is especially liable to be infected with trypanosomiasis.

Up to about fourteen, native boys are still children, after that they become men and work in the fields. After forty-five, as a rule, men have acquired a competence which allows them to rank as leaders in the direction, rather than in the execution of affairs. Up to twelve, girls are children, but after that they commence to spend the whole of their time in women's work. After forty, women are usually scarcely strong enough to do the hard work which falls to women of middle age. It is for these reasons that the divisions according to age have been made at the years specified in the table. An inspection of the table shows that approximately an equal number of males and females were examined.

There were eleven cases of trypanosomiasis in male children and five in female; but there were twenty-two cases of trypanosomiasis in adult males and twenty in adult females. There were no cases of trypanosomiasis in aged persons of either sex. These differences are not large ones, but they indicate that if any class in the Gambia is especially affected by trypanosomiasis it is that class which is most exposed, by reason of its occupation, to the bites of tsetse-flies.

Table III

Persons examined		Male 4714			Female 4355			Totals	
Age		0-14	14-45	45-X	0-12	12-40	40-X		
Size of glands	Seen	10	13	1	4	15	0	43	43
+	Punctured ...	3	12	0	2	10	0	27	
	Positive ...	3	12	0	2	10	0	27	
	Seen	45	22	0	25	24	2	118	118
+ —	Punctured ...	22	9	0	7	18	0	56	
	Positive ...	5	5	0	3	12	0	25	
	Seen	850	431	54	407	317	29	2088	2088
+ — —	Punctured ...	107	32	0	31	23	0	193	
	Positive ...	1	2	0	0	1	0	4	
	Seen	1119	1734	435	975	1927	630	6820	6820
—	Punctured ...	7	4	0	3	4	0	18	
	Positive ...	0	0	0	0	0	0	0	
	Totals ...	2024	2200	490	1411	2283	661		9069

Much the same result is obtained in Table IV, where all the cases of trypanosomiasis seen by us in the Gambia are classified in the same way, according to age and sex. For the purposes of this table all persons with ' + ' glands whom we were unable to puncture are considered to be infected. It is justifiable to do so because trypanosomes were found in all of the thirty-six ' + ' cases who were examined by gland puncture. It will be seen from this table that there are, still, more cases of trypanosomiasis among the male children, while the number of cases in adults is about

equal, and there are no cases in the aged. Results, very similar to these, were obtained by Kinghorn and Montgomery in Rhodesia.[11]

Table IV

Persons examined	Male			Female			
Age	0-14	14-45	45-X	0-12	12-40	40-X	Totals
Early cases	11	27	0	60	24	0	68
Clinical cases	2	4	0	0	5	0	11
Persons with + glands who were not examined ...	6	7	0	22	6	0	21
Total	19	38	0	88	35	0	100

Table V gives a list of the towns in which natives were palpated, together with the results of the examinations. Its interest is chiefly local; it may serve as a basis for future examinations undertaken to ascertain whether trypanosomiasis is increasing or decreasing in the Gambia.

A comparison of this table with the map shows that no district in the Gambia is especially affected by trypanosomiasis. Towns, such as Mandinaba, Demban Kai and Essau, which are placed near the water have, as is always the case in such towns, an unusually large percentage of their population infected.

Sometimes natives say that more persons die of sleeping sickness during the rains than in the dry season. This probably means only that more persons weakened by trypanosomiasis die of intercurrent infections at that time of the year because of the hardships caused by the unfavourable climatic conditions.

It is regrettable that lack of time and the reluctance of natives to be examined prevented the examination of all the ' + ' and ' + — ' cases. Many more ' + — — ' cases were seen than were examined; but the great majority of those whose cervical glands were puncturable have been examined. It would have been impossible to puncture a gland in the posterior cervical triangles of very many

TABLE V

	Towns	Number palpated	Degree of glandular enlargement +			Degree of glandular enlargement ± —			Degree of glandular enlargement + — —			Clinical cases	Probable percentage of Trypanosomiasis
			Seen	Punctured	Positive	Seen	Punctured	Positive	Seen	Punctured	Positive		
1	Dippa Kunda ...	41	1	0	0	4	2	0	12	0	0	0	2·4
2	Bakotti	45	0	0	0	11	3	0	18	2	0	0	—
3	Lamin	100	1	1	1	9	5	2	43	0	0	0	3
4	French Mission ...	10	0	0	0	0	0	0	3	3	0	0	—
5	Sukuta	100	0	0	0	5	0	0	30	0	0	1	1
6	Gunjur	100	0	0	0	4	4	0	24	4	0	2	2
7	Brikama	100	2	1	1	1	1	1	49	4	0	0	3
8	Sifa	54	0	0	0	13	0	0	21	0	0	0	—
9	Mandinaba	22	3	1	1	1	0	0	3	0	0	0	13·6
10	Tunjina	35	1	0	0	1	0	0	15	0	0	0	2·8
11	Pirang	50	2	1	1	1	0	0	18	0	0	0	4
12	Farraba Banta ...	110	1	1	1	6	6	0	62	4	0	0	0·9
13	Kafuta	77	4	4	4	3	3	0	40	1	0	0	5·2
14	Baijana	27	0	0	0	3	1	1	8	0	0	0	—
15	Somita	100	0	0	0	2	0	0	34	0	0	0	—
16	Demban Kaie ...	46	2	0	0	1	0	0	13	0	0	0	4·3
17	Kassang	25	0	0	0	1	0	0	9	0	0	0	—

TABLE V—*continued*

	Degree of glandular enlargement		I			II			III				
	Towns	Number palpated	Seen	Punctured	Positive	Seen	Punctured	Positive	Seen	Punctured	Positive	Clinical cases	Probable percentage of Trypanosomiasis
18	Vintang	100	0	0	0	5	2	1	30	3	0	0	1
19	Kuraukoto	9	0	0	0	0	0	0	4	0	0	0	-
20	Barong	109	0	0	0	1	1	1	28	1	1	0	0·9
21	Manduar	103	1	0	0	2	0	0	53	0	0	0	0·9
22	Jali	100	1	0	0	2	0	0	32	0	0	0	1
23	Batteling	120	1	1	1	2	2	1	49	3	0	0	1·6
24	Kwinella	152	0	0	0	2	2	1	45	1	0	0	0·6
25	Mandina	67	0	0	0	1	1	1	26	5	0	0	1·4
26	Kaiaf	130	0	0	0	2	2	1	43	4	0	0	0·7
27	Jappeni	79	1	1	1	2	2	2	26	3	1	0	5
28	Bureng	90	0	0	0	1	1	1	35	1	0	0	0·9
29	Sukuta	202	0	0	0	3	3	3	24	7	1	0	1·9
30	Brikama	107	1	0	0	4	0	0	39	0	0	0	0·9
31	Boraba	110	0	0	0	1	0	0	40	3	0	0	—
32	Sololo	66	0	0	0	0	0	0	38	4	0	0	—
33	Chakunda	84	0	0	0	1	1	1	28	1	0	0	1·2
34	Wellingara	62	1	1	1	0	0	0	21	5	0	0	1·6

TABLE V—*continued*

	Degree of glandular enlargement		—			+ —			+ — —				
	Towns	Number palpated	Seen	Punctured	Positive	Seen	Punctured	Positive	Seen	Punctured	Positive	Clinical cases	Probable percentage of Trypanosomiasis
35	Kumbe	140	5	0	0	0	0	0	35	0	0	0	—
36	Basse	125	2	0	0	1	0	0	48	0	0	1	2·4
37	Kulari	153	1	0	0	2	0	0	68	0	0	0	—
38	Tuba Kuta	63	—	0	0	0	0	0	16	1	0	0	—
39	Sunkunda	132	0	0	0	0	0	0	36	3	0	0	—
40	Kasi Kunda	64	0	0	0	2	0	0	26	1	0	0	—
41	Koina	194	2	2	2	0	0	0	70	15	0	0	1·03
42	Brifu	156	0	0	0	1	1	1	20	10	0	1	1·2
43	Bantonding	212	0	0	0	0	0	0	37	9	0	0	—
44	Sandi Keri	105	0	0	0	0	0	0	4	0	0	0	—
45	Hamedi Sirè ...	157	0	0	0	1	0	0	6	0	0	0	—
46	Maka	485	0	0	0	2	2	2	42	22	0	1	0·6
47	Makakaba	179	0	0	0	0	0	0	9	8	0	0	—
48	Kousalong	219	0	0	0	3	0	0	56	0	0	0	—
49	Karantaba Toranca...	55	0	0	0	0	0	0	12	5	0	0	—
50	Karantaba Tabokoto	115	1	1	1	1	1	0	14	7	0	0	0·8
51	Koli Kunda	100	1	1	1	0	0	0	22	3	0	0	1

TABLE V—*continued*

	Degree of glandular enlargement		+			+ —			+ — —				
	Towns	Number palpated	Seen	Punctured	Positive	Seen	Punctured	Positive	Seen	Punctured	Positive	Clinical cases	Probable percentage of Trypanosomiasis
52	Lamin Koto ...	124	1	1	1	0	0	0	18	0	0	0	0·8
53	Bandi...	125	1	1	1	0	0	0	12	0	0	1	1·6
54	McCarthy's Island ...	258	0	0	0	1	1	0	8	5	0	0	—
55	Kai-ai	182	0	0	0	2	1	1	41	5	0	0	0·5
56	Kuntaur	96	0	0	0	1	0	0	15	0	0	0	—
57	Gassan	54	0	0	0	1	1	1	9	0	0	0	1·8
58	Konkokoto	60	0	0	0	1	1	1	6	0	0	0	1·6
59	Jalokunda	212	0	0	0	0	0	0	44	5	0	0	—
60	N'Jaugen	19	0	0	0	0	0	0	3	0	0	0	—
61	Tento	129	0	0	0	0	0	0	28	4	0	0	—
62	N'Jau	242	1	1	1	1	1	0	25	4	0	0	0·4
63	Kau'ur	318	2	2	2	1	1	1	41	5	0	0	0·9
64	Ballanger	314	0	0	0	1	1	0	48	9	0	0	—
65	N'Geyen Sanjal ...	266	0	0	0	0	0	0	9	1	0	0	—
66	Medina	43	0	0	0	0	0	0	4	0	0	0	—
67	M'Bap	71	0	0	0	1	0	0	2	0	0	0	—
68	Sukotto	86	0	0	0	0	0	0	8	0	0	0	—

TABLE V—*continued*

	Degree of glandular enlargement												
	Towns	Number palpated	Seen	Punctured	Positive	Seen	Punctured	Positive	Seen	Punctured	Positive	Clinical cases	Probable percentage of Trypanosomiasis
69	Kai-ama	40	0	0	0	1	0	0	5	0	0	0	—
70	Dasilami	55	0	0	0	0	0	0	3	1	0	0	—
71	Faratenri	206	0	0	0	0	0	0	32	5	0	0	—
72	Yallol	35	0	0	0	0	0	0	0	0	0	0	—
73	Illiassa	311	0	0	0	1	1	0	27	5	1	0	0·3
74	Nokunda	252	0	0	0	0	0	0	29	6	0	0	—
75	Nja Kunda	200	0	0	0	0	0	0	23	7	0	0	—
76	Salikeni	167	1	0	0	0	0	0	40	7	0	1	1·2
77	Jammikunda ...	40	1	0	0	0	0	0	4	0	0	1	5
78	Kerewan	260	1	1	1	1	1	0	24	6	0	0	0·3
79	Dasalami	132	1	1	1	1	0	0	20	1	0	0	0·7
80	Bantanding	105	0	0	0	0	0	0	14	1	0	0	—
81	Daramé	52	0	0	0	1	0	0	0	0	0	0	—
82	Wabo	62	0	0	0	0	0	0	6	1	0	0	—
83	Dungku	70	0	0	0	0	0	0	10	0	0	0	—
84	Duniajoe (Mandingoe)	111	1	1	1	2	2	2	12	1	0	0	2·7
85	Duniajoe (Jolof) ...	31	1	1	1	1	1	1	3	0	0	0	3·2

TABLE V—*continued*

	Degree of glandular enlargement		+			+ —			+ — —				
	Towns	Number palpated	Seen	Punctured	Positive	Seen	Punctured	Positive	Seen	Punctured	Positive	Clinical cases	Probable percentage of Trypanosomiasis
86	Essau	111	6	5	5	1	0	0	14	0	0	0	5·4
87	Bathurst	1518	7	5	5	7	5	2	*	11	0	1	0·6
88	Bakau	176	2	1	1	1	0	0	7	0	0	0	0·5
89	Waslunga	86	0	0	0	0	0	0	8	0	0	0	—
90	Jessuan	32	0	0	0	0	0	0	1	0	0	0	—
91	Tarancakunda ...	50	1	0	0	0	0	0	6	0	0	0	2
92	Latikunda	90	0	0	0	0	0	0	3	0	0	0	—
93	Sarakunda	39	0	0	0	0	0	0	0	0	0	0	-
94	Mangai Kunda ...	39	0	0	0	1	0	0	4	0	0	0	—
95	Kokoli	27	0	0	0	0	0	0	2	0	0	0	—
		12298	56	36	36	136	63	28	2102+	233	4	11	
			0·4%			1·1%			17%				

* No record was kept of the ' + — — ' cases seen in Bathurst.

of those who have been classified as ' + — —'. Four ' + — —' cases whose neck glands were too small to be punctured were punctured in other groups; trypanosomes were found in none of them. Neither were parasites found in three cases whose neck glands were much enlarged from tuberculosis and contained necrotic material.

G. *Pulse and temperature.*

It has been noticed frequently that a rapid pulse and a slight degree of occasional fever are often early symptoms of human trypanosomiasis. It was, consequently, thought that the presence of both, or either, might be of value in encouraging the search for trypanosomes in early cases of trypanosomiasis in whom the parasites could not be found at a first examination. The pulse rates and temperatures were, therefore, taken in 281 persons whose gland juice, or blood, was examined for trypanosomes. Cover-slip preparations, smears, and thick films were made from the blood of all these persons. It soon became evident that an increased pulse was of little value because the nervousness of the natives on account of the examination often ran up the pulse rates, in even healthy adults, to 100, or more. This was especially true of the children; some of them had never before seen a white man. Our records show many children, and a few men and women, who have normal or only slightly elevated temperatures, and pulse rates of 120 or even 130 beats to the minute. Examination of the blood has shown that some of these persons had malaria; we believe that in most of them the rapid pulse was simply caused by apprehension. As a rule, there was a distinct increase in the pulse rate of the sixty-one infected persons examined to 100, 120, or more, and that with temperatures of only 99° F. This was not always the case, for one man of thirty-five, with trypanosomiasis, had a pulse rate of 63 and a temperature of 99° F. when he was first seen; others had pulse rates of 78, with temperatures between 99° F. and 100° F.

It has long been known that an isolated observation of an abnormally high temperature in a case of trypanosomiasis does not bear a very definite relation to the probability of finding trypanosomes in the peripheral circulation of that case. In the present series there was, almost always, a slightly elevated

temperature, between 99° F. and 100° F. in those in whom trypanosomes were found. Two persons who had no malarial parasites in their blood, had temperatures of over 100° F.; trypanosomes were seen in the blood of only one of them. Apparently healthy persons, especially young adults and children, were often seen in whom neither malaria parasites, trypanosomes, nor any obvious cause for fever could be found, who had temperatures varying between 99° F. and 100° F. We have no explanation to offer. We do not think that the natives' temperatures were inaccurately recorded because of the high temperature of the air which often reached 105° F. or 107° F. The thermometer was always carefully cooled to below 96° F. with water before being used; oral temperatures were always taken, and the native was made to keep his lips tightly closed so long as the thermometer was in his mouth.

VI. THE METHODS OF DIAGNOSING TRYPANOSOMIASIS DISCUSSED AND COMPARED

One object in initiating a comparative series of examinations, by different methods, for the presence of trypanosomes in a series of 283 persons, was not so much to determine which was the most efficient method, but which was the most effectual. It was not wished to determine by which method the largest number of cases could be detected by spending unlimited time in repeated examinations. It was wished to determine which method would discover the largest proportion of cases in the shortest time. For that reason a limit was set on the length of time to be spent in examining preparations made by each of the methods employed.

One cover-slip preparation of blood and one of gland juice were usually examined from each case; if more were examined the fact has been noticed in the comparison of results. About fifteen minutes are required in which to properly examine a $\frac{3}{4}$ inch square cover-slip preparation of blood for living trypanosomes; the same length of time is needed for examining a similar preparation of gland juice. Preparations of gland juice can be perfectly well stained first and examined later for trypanosomes; all of ours were examined in fresh cover-slip preparations because we believe it to be the easier and the quicker method; we have never examined gland juice in hanging drops.

Because the examination of fresh preparations requires only fifteen minutes, it was determined to spend only fifteen minutes in searching a smear or a thick film. This limit has been rigidly observed in all our cases save in the examination of preparations coming from cases in whom trypanosomes had been found by gland puncture. These were searched, unless trypanosomes were found sooner, for half an hour at least.

Table VI

	Gland Puncture		Thick Films		Thin Blood smears		Coverslip preparation	
	Single examination	Repeated examination	Single examination	Repeated examination	Single examination	Repeated examination	Single examination	Repeated examination
Trypanosomes found ...	48	2	7	1	9	0	5	1
Total ...	50		8		9		6	

This table is a record of the simultaneous examination of 283 persons, selected by gland palpation, by gland puncture, by thick blood films, by thin blood smears, and by fresh coverslip preparations of blood. The successful examinations made by each method are sub-divided so that it can be seen whether a more severe examination than that ordinarily used was employed; it must be understood that, usually, more than the allotted length of time was spent in searching for trypanosomes by each method when they had been found previously by some other method.

Because of the great length of time which it requires, attempts to centrifugalise the blood were soon abandoned, and the only methods used in each of the 283 cases, compared in Table VI, were a cover-slip preparation, a smear, a thick film of blood, and the examination of a fresh cover-slip preparation of gland juice. An inspection of this table proves that, in the Gambia, gland puncture is by far the most successful of the methods we compare. It is consequently a very valuable diagnostic method. It is doubly valuable because of its simplicity and because of the rapidity with which it can be employed. The chief value of smears and of thick films of blood is that they provide a means by which specimens, taken when there is no opportunity of examining them, may be

preserved to be searched for trypanosomes later on. Since, at least, three or four times the amount of blood required for a smear is used in making a thick film, parasites certainly ought to be found more often in thick films than in smears. Some observers have found thick films more useful than gland puncture in finding trypanosomes in some cases, especially in those cases from whom the parasites have disappeared after treatment. We have very little confidence in this method. A trypanosome may lie, unrecognisable, at the bottom of some of the heaps of débris which remain, and stain, in every thick film. Many of the trypanosomes found in thick films are only partially stained, sometimes almost nothing of the parasite can be seen beyond a nucleus and a blepharoplast. A trained microscopist who has examined many thick films may always be able to recognise such trypanosomes quickly, others can not. The time required for the preparation of a thick film is also an objection to the method. All of these reasons led to the abandonment of the dehaemoglobinised thick film method when it was first tried by us in 1903. We were searching then for a rapid routine method of recognising trypanosomiasis and we decided that the centrifugalising and fresh examination of small quantities of blood was a much better method of examining it. At that time, it was also decided, from the examination of a very considerable number of smears and cover-slip preparations, taken at the same moment, from animals and men infected with trypanosomes, that the parasites could be found more easily, living and moving, in fresh preparations than dead and motionless in stained ones. Attempts to make living trypanosomes, in cover-slip preparations of blood, more conspicuous by vital staining were also abandoned, among other reasons, because most aniline dyes evidently hastened the death of the trypanosomes; neutral red was the least harmful of those which we tried. In view of our belief that the examination of cover-slip preparations is a better method than the examination of smears, the figures given for each method in Table VI and in the paragraph describing the findings obtained by the examination of cover-slip preparations are surprising to us.

Gland palpation is very simple. Large neck glands can be recognised by any intelligent negro, and the persons possessing them can be brought to a doctor for examination by any native

policeman. Gland puncture is not a difficult manoeuvre. With the assistance of an orderly, capable of boiling a syringe, gland puncture can be done and the whole of the specimen examined in less than twenty minutes. From our experience in the Congo, in Sierra Leone, and in the Gambia, we can only conclude that gland palpation, followed by gland puncture is by far the most effectual means of finding trypanosomes in, at least, those early cases of the disease seen by us.

The shortcomings of gland palpation and puncture have always been very evident. Very early cases in whom glandular enlargement has not appeared, and late cases from whom it has disappeared may be missed by it; the missing of the latter group of cases is not serious since they are usually easily recognised by gross clinical signs. The missing of the first group is serious. At present there is no means of determining how large a proportion of the cases of trypanosomiasis present in a community will be missed by gland palpation and gland puncture; that cases will be missed is abundantly shown by work done in the Congo[2]. It was shown there that a man without enlarged glands might have trypanosomiasis in his cerebro-spinal fluid, although he seemed quite healthy and although parasites were not found in his blood; it was also shown that trypanosomes might not be found in the gland juice of a small percentage of persons although they were present in the finger blood. Many observations made since then have shown the same thing; but even if an appreciable percentage of cases is missed by this method that is no reason why the method should not be used in attempts to check the disease by the restraint and treatment of the exceedingly considerable percentage of cases which can be detected by it. It is difficult to estimate the number of cases of trypanosomiasis which will remain undetected by gland palpation and gland puncture, because there is no certain method of recognising the disease. Its absolute diagnosis rests upon the demonstration of the parasite causing it and, in our hands, the method of examination which we wish to control has been much the most efficient of all the methods at present available for finding trypanosomes; until more perfect means of diagnosis are devised, the only certain way of determining what proportion of cases of trypanosomiasis are missed by gland palpation and

puncture would be to keep a substantial number of persons who have been examined by the method under observation for a considerable period in a locality where they would not be exposed to re-infection and where it would be possible to re-examine them at intervals. The Gambia does not offer these conditions perfectly, but it does so to a considerable degree; and it is hoped that the results of future observations, made on those persons, with slightly enlarged glands, in whom we found no trypanosomes, may make it possible to form an estimate of the proportion of infected persons whom the examination of glands failed to detect there. It was attempted to keep track of the natives with enlarged glands who were examined in the Congo[3]. It is impossible to draw any certain conclusions from the reports which have been received concerning them, because many of them are mising, and because they inhabited areas where sleeping sickness is endemic. Nevertheless, an examination of the figures suggests that a larger number of those with enlarged glands, in whom trypanosomes were either not found or not looked for, ultimately died of sleeping sickness than would have been expected had they been entirely healthy persons.

We do not anticipate that the proportion of infected persons in the Gambia in whom trypanosomes have not been found by gland palpation and puncture will be a large one, if only for the following reasons. The efficiency of gland examination depends upon the selection of persons with enlarged glands for puncture. All of those with much enlarged ' + ' glands are almost always infected; in the Gambia trypanosomes were found in thirty-six out of thirty-six. Parasites are frequently found in those with moderately enlarged ' + — ' glands by a single examination (in the present instance twenty-eight out of sixty-three were infected); if such persons were detained for examination they would be examined, frequently, over a period of some weeks before being allowed to proceed[2]. It is very probable that, in this way, some of them would be shown to be infected. Always, very few cases are found among those with glands that are very slightly enlarged, ' + — — '; in the Gambia there were only four cases among 233 persons examined (see Table II). All four of these cases were persons who had moderately enlarged ' + — ' glands on one side of their neck; consequently, if they had been detained as

suspected cases, they would have been repeatedly examined before being passed as probably uninfected persons. If these four cases be subtracted, 229 persons with little more than normal glands remain; their glands were punctured and their blood was examined in cover-slip preparations, in smears and in thick films. None of them were found to be infected. Of course the result would have been more convincing had the examination been often repeated, if the blood had been centrifugalised, and if susceptible animals had been inoculated from all these persons; but the examination which they did receive was not an entirely insignificant one, and we believe that the immediate future will not show that many individuals among these 229 persons had trypanosomiasis when they were examined.

The glands of two persons, one a ' + ' case, the other a ' + — ' case, in whom trypanosomes were found by examining the blood, were not punctured; it is probable that gland puncture would also have detected the parasites. In one instance trypanosomes were found in a blood smear from a ' + — ' case in whom parasites were not found by a single perfect examination of gland juice. We regret that most of our blood smears and thick films were not examined until our return to England, and that it was consequently impossible to examine these persons by gland puncture until we were satisfied that trypanosomes could be found in them by this method of examination.

It must be remembered that trypanosomes can be found in the lymphatic glands of any group, and that the femoral or axillary glands can often be punctured when those of the posterior cervical triangles are too small to be examined. The continued observation of a series of suspected or actual cases of trypanosomiasis, in whom trypanosomes can not be found by gland puncture, is greatly needed; it might throw interesting light upon the course and development of the disease.

The use of gland palpation and puncture was first urged as the basis of measures intended to check the spread of trypanosomiasis because of figures obtained, in the Congo Free State, from the simultaneous examination by different methods of several hundred persons; 250 of them were cases of trypanosomiasis. The work which led to these results was the direct outcome of an observation

made by Greig and Gray, that trypanosomes were present in the glands of persons with trypanosomiasis. We had long been searching for a rapid method of diagnosing the disease, and the idea at once presented itself that such a method might be found in gland puncture[17]. In the hope of finding a part of the body in which the trypanosomes occurred in larger numbers than in the blood, a series of preparations had been taken immediately after death from the organs and body-fluids of many persons and animals; in only one case, examined within half an hour after death, were living trypanosomes, found in the glands[25]. This observation is an interesting one, since it emphasises, and may have some relation to the fact that trypanosomes sometimes die very quickly in preparations of gland juice. Consequently, preparations obtained by the puncture of glands must be examined as soon as they are made.

Methods exactly similar to those used in the Congo have been employed by us, or by one of us, in the Gambia and in Sierra Leone with the same results. Similar results have been obtained by many persons in many parts of Africa; yet, it is true that gland palpation and puncture have failed to detect cases of trypanosomiasis in Ashanti, on the Gold Coast, in Nyasaland[18], and elsewhere. As far as the Nyasaland cases are concerned it will be interesting to observe whether human trypanosomiasis there, which is said not to be caused by that parasite, runs the same course as the disease produced by *Trypanosoma gambiense*.

VII. THE PRACTICAL APPLICATION OF GLAND PALPATION AND PUNCTURE

Although we have urged, and do still urge, that gland palpation and puncture should be made the basis of measures enforced, in areas, where human trypanosomiasis exists or threatens to become endemic, with the object of collecting natives for treatment and isolation in some type of restricted settlement; we do not, in any way, urge that this method should be used in the maintenance and administration of such measures to the exclusion of all other methods of diagnosing trypanosomiasis. It is a very efficient method of diagnosis; in our experience it is the most efficient one.

It is almost the quickest of the methods in individual examinations, and by it a large percentage of infected persons can be weeded out from a whole population infinitely more quickly and more cheaply than can be done in any other way. It is possible that other methods might detect a slightly larger percentage of infected persons, but economic exigencies—expense—will frequently make it impossible to employ enough trained physicians to carefully examine every native in a district by the tedious, though possibly more efficient, methods of centrifugalisation of the blood; the expense of maintaining a staff of physicians and native assistants capable of examining the native population in that district by gland palpation and puncture would be very much less.

No one can deny that gland palpation and puncture form a very efficient method of diagnosing trypanosomiasis; in our hands it has been the most efficient of all methods, although it does sometimes fail. It would be regrettable if the extravagant claims which have been made for the method should lead to a reaction by which its real value might be obscured.

In our experience it has not be a difficult method to employ. Natives whose glands are punctured feel nothing after the prick of the needle and, which is almost as important, they can see nothing. Once or twice whole villages have become frightened and refused to be examined; but that has been very unusual. Tact and a generous distribution of sweets, beads, kola nuts and small novelties has usually successfully overcome all distrust[14].

In practice, we believe that much can be done in many parts of Africa to control trypanosomiasis if measures suggested by a knowledge of the incidence of the disease are enforced. That knowledge, in our opinion can be gained most quickly and easily by gland palpation and puncture. Just as the French Commission, the German Commission, Broden and Rodhain and others have observed, it was seen in the Congo that some cases—especially very early ones and late ones—are missed by the examination of single preparations of gland juice, or of even several preparations taken during a period of a few days. Consequently, when it is possible, gland palpation and puncture must be supported by other, though less efficient, methods.

The presence of auto-agglutination, of increased pulse-rate and

of heightened temperature in persons, apparently otherwise healthy, will always be suspicious and, as is suggested by the paragraphs dealing with these signs, be sufficient to cause the examination of persons possessing them to be persevered in. But, none of these signs have any diagnostic value in themselves. The value of examining the blood for trypanosomes by inspection and by animal inoculation is admittedly very great, and these devices must be employed where it is necessary to do so.

In our opinion the most convenient and most effectual order for the routine examination of persons for trypanosomiasis is gland palpation and puncture, cover-slip preparation of blood, centrifugalisation of the blood, thick blood films, blood smears and animal inoculations. Auto-agglutination and clinical signs are valuable, but they are merely suggestive.

VIII. NATIVE TOLERANCE OF TRYPANOSOMIASIS

An inspection of Tables II and IV shows that we saw no cases of enlarged glands, nor of trypanosomiasis, in persons past middle age. In reviewing these tables, it was suggested that this absence of cases might be explained in part by the lack of exposure to infection of those past middle age, because of the occupations which they follow; but, it may be that this lack of cases is due to an immunity acquired later in life. The idea is not a new one[1, 14, 21].

The fact that trypanosomiasis has been present in the Gambia and elsewhere on the West Coast of Africa for many years, in places where *Glossina palpalis* exists, without assuming the epidemic form which it has taken in the Congo Free State and in Uganda, of itself, suggests that the West Coast natives may have acquired some immunity to it. In the Gambia the customs of natives, almost none of whom were riverine, has doubtless much to do with preventing the spread of trypanosomiasis; but there seems to be something more than that.

There are many records, some based altogether on the observations of Europeans, others based on observations of natives and of Europeans, of persons who have lived for four or more years after they probably became infected by trypanosomes; these records prove that persons may have a 'tolerance' for trypanosomes

and live comparatively healthily though infected by them. This fact must be remembered in appreciating the results of treatment, for it must be asked whether an improvement in the condition of a patient has been due to the drug administered or whether the treatment has merely coincided with the commencement of a period of tolerance in which the parasites are not necessarily destroyed, although the effect produced on the patient by them almost disappears.

Little is known of the reasons which may cause trypanosomiasis to assume an acute phase. In experimental animals it is known that an intercurrent infection may determine a sudden multiplication of trypanosomes. It may be that some similar factor which 'produces a lessened resistance' might be the cause of an acute phase of trypanosomiasis in persons who had previously had a chronic form of the disease.

Almost nothing is known of the outcome of those cases of trypanosomiasis in whom parasites are present, although there are almost no symptoms. It is not known whether they recover, nor, if they die, for how long the disease may run a chronic course.

It may be suggested that the overwhelming preponderance of middle-aged persons in our infected cases might be explained by the chronic nature of the infection which allowed those infected in youth to live on to middle-age, still infected, but tolerant of their infection. In that case the absence of cases after middle-age might be the expression of an immunity acquired as the result of a preceding persistent infection.

Observations on these points are needed badly; it is to be hoped that many of those who were found to be infected in the Gambia by this expedition can be followed. It is worth noting that all of the six persons who were found to be infected in the Gambia in 1902 were dead in 1906.

IX. RECOMMENDATIONS

It is not our intention to propose a scheme for the prevention of sleeping sickness in the Gambia. To do so is the province of the Resident Medical Officers, and it is they who will decide which of the measures, which have been employed elsewhere in the prevention

of sleeping sickness, are most applicable to the situation in the Colony of the Gambia.

There are, however, a few very evident improvements to be made which may be mentioned. The situation of the town of Essau, where five per cent. of the population are infected with trypanosomes should certainly be changed. The town is at present placed at the side of a swamp and is almost enclosed on two sides by mangroves. As a result *Glossina palpalis* is often seen within the village. Demban Kai, where 4·3 per cent. of the population are infected is situated almost as badly. It should be moved. Mandinaba where three out of twenty-two persons were probably infected, might, also, be moved.

There are many fords and river-side washing places which are closely enclosed by mangrove bush and by other thick vegetation. Tsetse-flies are always present in such places; consequently the natives who frequent them are constantly bitten, and it would be well if all the thick brush about fords, wharfs, or places used for washing by women, could be cleared for a distance of at least 150 yards on every side. The strip of bush just to the south of the town of Bakau, at Cape St. Mary, swarms with *Glossina palpalis*. The thick undergrowth should be cleared from this bush, and that especially if it is determined to establish permanent European quarters at the Cape. At present this bit of jungle is a menace to the neighbouring native town and to everyone who passes through it.

The growing of rice should be forbidden in places where the fields are in swamps near the river, where *Glossina palpalis* exists; most of the rice-fields in the Gambia are of that sort. It is possible that 'mountain rice' (*Oryza sativa*, var.), which will grow in comparatively dry places, might be substituted in the Gambia for the present variety, which can only be grown in marshes. Even if 'upland' or 'mountain rice' cannot be grown, the prohibition of the cultivation of marsh rice would not be a great hardship. Many natives, for example, those to the north of the Niumi Forest, eat no rice and they, like many others, look on it as a luxury rather than a necessity. Many of those who grow rice have eaten it all within three or four months of the harvest time, and for the rest of the year live on other grains. Others eat it regularly twice, or even

three times a week. Very few of the towns use maize as much as they might do and, possibly, it might be substituted for rice extensively.

We believe that much can be done to improve the situation in the Gambia by such methods and by the isolation and treatment of infected persons who would be detected, largely by systematic gland palpation of the whole population. There is very much in the description of the preventive measures employed in the Congo Free State which seems to be good (Sleeping Sickness Bulletin, No. 26, p. 193). The clauses which forbid the concealment of cases, make notification of cases compulsory, and make traders and other employers of labour responsible if infected persons with enlarged glands are found among their employés, seem to be especially praiseworthy.

The examination of the population and the treatment and maintenance of the cases detected would be placed under the direction of, probably, two medical officers appointed for that purpose and assisted by native orderlies and inspectors. From our talks with the natives, we do not think that it would be very difficult to persuade them to send cases of trypanosomiasis, at least those in whom symptoms are evident, to villages established for observing and treating them. It would be comparatively easy to get the natives to come to them if two such villages were established in the Gambia, one near the sea coast, and a second up the river, probably at McCarthy Island. The establishment of such villages would be worth while, if only for the sake of the observations which it would be possible to make from them on the course of the disease in persons sent to them. It would be worth while also, from the patients' point of view, for it does seem certain that, in some instances, early, radical and persevering treatment may cure trypanosomiasis.

The physicians attached to the sleeping sickness isolation villages would have opportunities for studying many interesting points which can only be investigated by those resident in the Colony for considerable periods. Not the least of these would be the careful examination of a large number of native and wild animals in order to ascertain whether *Trypanosoma gambiense* occurred naturally in any of them.

X. CONCLUSIONS

1. Gland palpation and puncture is, by far, the most effectual of the procedures, employed by us in the Gambia, for the diagnosis of human trypanosomiasis.

2. At least 0·8% of the population of the Gambia are infected with trypanosomes.

3. Measures designed to control human trypanosomiasis may be usefully instituted in the Gambia; they should include a continued examination of the whole population, the establishment of villages for the isolation, observation, and treatment of cases, and the appointment of a special staff for the administration and execution of these projects.

XI. REFERENCES

1. Dutton and Todd. First Report of the Expedition to Senegambia. Memoir XI of the Liverpool School of Tropical Medicine, 1902.
2. Dutton and Todd. Gland Palpation in Human Trypanosomiasis. Memoir XVIII of the Liverpool School of Tropical Medicine, 1906.
3. Todd. A review of the position of Gland Palpation in the Diagnosis of human trypanosomiasis. Journal of Tropical Medicine and Hygiene. August 1, 1908.
4. Hopkinson. Sleeping Sickness Bureau Bulletin. No. 21, p. 381.
5. Diagnosis of Human Trypanosomiasis. Bulletin of the Sleeping Sickness Bureau, No. 2, p. 53. Sleeping Sickness Bureau, Royal Society, Burlington House, London, W., England.
6. Report on Human Trypanosomiasis from Leopoldville. Bulletin No. 5, p. 190.
7. Symptoms of Human Trypanosomiasis. Bulletin No. 9, p. 330.
8. Report of the Commission to April, 1909. Bulletin No. 10, p. 397.
9. Report of the Commission 1906-1908. Bulletin No. 10, p. 355.
10. Report of the Commission 1906-1907. Bulletin No. 11, p. 420.
11. Second Report on Human Trypanosomiasis in North-Eastern Rhodesia and Nyasaland. Bulletin No. 12, p. 467.
12. Diagnosis of Human Trypanosomiasis. Bulletin No. 14, p. 57.
13. Nyasaland Sleeping Sickness Diary. Bulletin No. 17, p. 181.
14. Report on Sleeping Sickness in the Volta District. Bulletin No. 18, p. 222.
15. Sleeping Sickness News from the Gold Coast. Bulletin No. 19, p. 257.
16. Sleeping Sickness News, Gold Coast. Bulletin No. 21, p. 378.
17. Dutton and Todd. Gland Puncture in Trypanosomiasis. Memoir XVI, Liverpool School of Tropical Medicine, 1904.
18. Sleeping Sickness News from Nyasaland. Bulletin No. 22, p. 415.
19. Report on Sleeping Sickness in Northern Rhodesia, 1910. Bulletin No. 23, p. 36.
20. Report of the Sudan Sleeping Sickness Commission. Bulletin No. 24, p. 85.
21. Report on Human Trypanosomiasis in Ashanti. Bulletin No. 25, p. 133.
22. Kinghorn and Montgomery. Report of the Sleeping Sickness Expedition to the Zambesi, 1907-08. Annals of Tropical Medicine and Parasitology, Vol. II, No. 2.
23. Sleeping Sickness News, Northern Nigeria. Bulletin No. 5, p. 204. See also Bulletin No. 21, p. 385.
24. Yorke. Auto-agglutination of Red Blood Cells in Trypanosomiasis. Bulletin No. 24, p. 53.
25. Dutton, Todd and Christy. Human Trypanosomiasis in the Congo. Memoir XIII, p. 42, 1904.
26. Kinghorn. Bulletin No. 16, p. 147.



The route followed by the Expedition is indicated thus, ——.
The names of towns where natives were examined are underlined.
16°30'
16°0'
15°30'
15°0'
13°30'
13°0'
NIUM-BATO
Cape St Mary
Dense Forest
Bamboo Forest
Sabkeni
Kerewan
Battelling
Kwinella
Medina
Jau Bambali
Kanuma N' Basen
Mula Point
Jah Point
Karantaba
Kombo Forest
Katamina



14°30'
14°0'
14°0'
13°30'
13°0'
Konantu
Kodiamo
Kuntuata
Katella
Sare Dembari
Demba Kunda
Bamboo Forest
Taba Nani
Changoi
Manna
Jamaris
Karantaba
Kussalang
Bansang
Sandugu Mana
Sao Tenda
Pakeba Alfa Maka Sise
Pakeba
Waha
Bamboo Forest
Koli Kassa
Duta Bos
Baro Kunda
Bantanting
Sare Amadu Sira
Tabanding
Taarra
Naude
Sare Kuba
Nankun
Kuran
Diabugu
Kaudonoli
Jakabu
Yoruba
Tarabussan
Fatato
Kerewan
Fula Tenda
Sofi Kunda
Bantanto
Bamboo and Small Trees
Julu Kunda
Koro Nunu Kunda
Sare Boja Demma
Bamboo Forest
Bakadaji
Madina
Sare Kolsa
Sare Nialo
Sare N'Galam
Lenkel
Sare Dumbel
Gambissara
Sare Biru
Nema Tuba
Bantanta
Willingara
Nello
Kumbali
Kurop
N'Galias
The Upper Valley of the Casamance R.
Sare Gide
Manakaba
Tabajan
Sandi Kunda
Sami Kunda
Kanape
Kusai
Willingara
Bantunding
Kaina
Kenniba
Jau Kunda
Kasi Kunda
Walli Kunda
Bamboo Forest and Small Trees
Gambissara
Basse
Basse
Fula Mani
Badara
Sise Kunda
Bamboo Forest
Netebulu
Malinjan Kunda
Jamoto
Tabajan
N T O R A
S A N D U
W
K A N T O R A
U L
A D U
G U

THE MECHANISM OF THE PRODUCTION OF SUPPRESSION OF URINE IN BLACKWATER FEVER

BY

WARRINGTON YORKE

AND

RALPH W. NAUSS

(From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine).

(Received for publication 17 June, 1911)

Suppression of urine is a by no means infrequent sequel to an attack of blackwater fever. Owing to the extreme gravity of this symptom—responsible as it is for a great proportion of the fatal cases—the mechanism of its production is a subject of considerable practical importance. It is unnecessary here to refer in detail to the work of previous authors, as a full account of the bibliography of the subject is given in the papers of Werner* and Barratt and Yorke†. The work of these authors and of de Haan‡ upon the condition of the kidneys of blackwater fever patients who have succumbed from suppression of urine has demonstrated the existence of granular material in the lumen of the renal tubules, and these workers infer that the suppression of urine is of mechanical origin, dependent upon the occlusion of the tubules by granular plugs. On the other hand, a nervous inhibition of glomerular secretion is regarded by A. Plehn§ as the essential factor in this condition. Again, nephritis has been mentioned by some writers as playing an important part in its production.

* 'Ueber die Nieren beim Schwarzwasserfieber,' Archiv. für Schiffs- und Tropenhygiene, 1907, Band XI, Bieheft 6.

† 'An investigation into the Mechanism of Production of Blackwater,' Annals of Tropical Medicine and Parasitology, 1909, Vol. III, No. 1.

‡ 'Die Nieren beim Schwarzwasserfieber,' Archiv. für Schiffs- und Tropenhygiene, 1905, S. 22.

§ 'Die Nieren beim Schwarzwasserfieber,' Archiv. für Schiffs- und Tropenhygiene, 1903, S. 270.

For many years it has been known that the injection of such poisons as glycerine, pyrogallic acid, toluylen-diamin and haemolytic serum frequently produces haemoglobinuria and sometimes leads to suppression of urine. It is doubtful whether the condition produced by such injections can be regarded as at all comparable with that occurring in blackwater fever, since it is highly improbable that the poisonous effects of the drugs in question are limited to the red blood cells.

Barratt and Yorke* observed that intravenous injection of an isotonic solution of haemoglobin made from the animal's own erythrocytes was quickly followed by the appearance of haemoglobinuria. The injections resulted in an increased flow of urine. Sections of the kidneys of these animals showed the presence of a certain amount of granular material in the lumen of some of the tubules. In none of the animals was there any tendency towards anuria.

Whether the occurrence of haemoglobin free in the blood plasma and its subsequent elimination by the kidneys is sufficient to produce suppression of urine through mechanical blocking of the renal tubules, or, whether a nephritis, or nervous inhibition of glomerular secretion, is to be regarded as the essential factor in the development of this condition in blackwater fever is a question, therefore, which has not, as yet, been definitely decided. It was with the object of throwing further light upon this point that our investigations were undertaken.

Our experiments were, with certain modifications, carried out along lines similar to those employed by Barratt and Yorke, i.e., the production of experimental haemoglobinuria in rabbits by the intravenous injection of solutions of homologous haemoglobin.

Technique. An isotonic solution of haemoglobin was made as follows:—A rabbit was bled from the external jugular vein and the blood collected in oxalated saline solution. The red blood cells, separated from the plasma by centrifugalisation, were washed three times in normal salt solution and then laked by the addition of distilled water. Sufficient sodium chloride was now added to render the solution isotonic and the precipitated stromata thrown down by means of the centrifuge. The clear solution of

* *Loc. cit.*

haemoglobin was then withdrawn. The percentage of haemoglobin present in the solution was estimated, in terms of human red blood cells, by means of a von Fleischl haemoglobinometer. As it was undesirable to bleed the experimental animal to an undue degree, the haemoglobin solution was prepared in part from the animal's own red blood cells, and in part from those of other rabbits. In order to facilitate the collecting of specimens of urine the bladder was opened supra-pubically, a cannula inserted and the wound closed by sutures. The rabbit was then placed in a specially constructed box which allowed of moderately free movement, but was sufficiently narrow to prevent the animal from turning round. A rubber tube attached to the glass cannula passed through a hole at the end of the box into a capsule in which the urine was collected. The solution of haemoglobin, warmed to 37° C., was now slowly injected into one of the veins of the ear. After an interval of two or three minutes to allow of the haemoglobin solution being thoroughly distributed throughout the circulation, a sample of blood was removed from the other ear and the degree of haemoglobinaemia estimated. The urine in the cannula was observed to be tinged with haemoglobin a few minutes after the injection. From time to time the urine was removed from the capsule, its volume measured and the amount of haemoglobinuria estimated.

Our earlier attempts to produce anuria by this means were invariably unsuccessful. Injection of the haemoglobin solution was followed by an increased flow of urine. This was probably to be explained by the diuretic action of the sodium chloride solution in which the haemoglobin was dissolved. After a time the diuresis subsided, but, as a rule, it lasted until the haemoglobinuria was distinctly decreasing in amount.

In later experiments we endeavoured to reproduce more closely the conditions obtaining in blackwater fever in man. In this disease the blood is anaemic and the patient is more or less collapsed. It appears probable that a decreased filtering force in the glomeruli, resulting from a low blood pressure, may play a not unimportant part in the production of anuria in these cases.

With this consideration in view we decided to reduce the blood pressure of our experimental rabbits, partly by keeping the animals

on as dry a diet as possible for twelve hours previous to the injection of haemoglobin, and also during the experiment, and partly by bleeding the animal to a moderate degree from the external jugular vein.

In order to obviate as far as possible the diuretic action of the sodium chloride, the strongest obtainable solution of haemoglobin was employed. Such solutions were prepared by dissolving the washed erythrocytes in the minimum quantity of distilled water necessary to produce complete laking.

As a result of these precautions we succeeded in producing suppression of urine in a number of animals.

Condition of the blood during experiment. Only a trace of haemoglobinaemia was detectable in the blood of the animals at the commencement of the experiment before injection of the haemoglobin solution. As a rule, bands of oxyhaemoglobin were just visible in a column 20 mm. high corresponding to an amount of haemoglobin equal to less than 0·1 per cent. The amount of haemoglobin injected into the circulation at one time varied from 1·5 to 13 grammes. Examination of the blood from time to time showed that the amount of haemoglobinaemia gradually decreased. The rate of disappearance was not constant, however, but occurred more rapidly at first and then gradually became slower, whilst the last traces of haemoglobin solution persisted for many hours. Moreover, it was occasionally observed that when a second or third injection of haemoglobin solution was made in the same animal after an interval of six to twelve hours a high degree of haemoglobinaemia persisted for a considerably longer period than that resulting from the first injection, even though a greater quantity of haemoglobin was introduced on this occasion than on the second. This point is well illustrated in Table 11, where, after the first injection the haemoglobinaemia within five hours fell from 12·6 to 2·8 per cent., whilst, after the second injection it only decreased from 9·7 to 5·25 per cent. during a period of nearly sixteen hours.

The significance of this observation is not very obvious. As only a comparatively small proportion (Table 1) of the haemoglobin circulating in the plasma is eliminated through the kidneys into the urine, the greater portion must be broken up in the

body. It has been shown by Tarchanoff* that the intravenous injection of solutions of haemoglobin in dogs is followed by a considerable increase in the formation of bile pigment. Moreover, the work of Simpson† has demonstrated that in those cases of malaria in which there is reason to believe that considerable destruction of red blood cells is occurring, the amount of faecal urobilin is greatly increased. It appears probable, therefore, that

Table 1.—Comparison of the amount of haemoglobin injected intravenously with that excreted in the urine.

No. of Experiment	Amount of haemoglobin injected intravenously g.	Amount of haemoglobin excreted in urine g.	Ratio of amount injected to amount excreted in urine	Remarks
1	3·4	0·72	1 : 5	Urine contained haemoglobin when animal died.
2	16·2	0·97	1 : 5	,,
3	29·4	5·3	1 : 6	
4	29·8	6·34	1 : 5	
5	16·0	1·45	1 : 11	
6	41·0	2·8	1 : 15	
7	23·7	1·15	1 : 21	Suppression of urine
8	28·2	2·66	1 : 11	,,
9	20·8	1·5	1 : 14	,,

a large proportion of the haemoglobin introduced into the circulation is got rid of by the liver, and that after a time the liver cells lose to a certain extent their capacity for eliminating from the blood stream large quantities of haemoglobin, and hence the rate of disappearance of haemoglobinaemia may be in the later stages much retarded.

* 'Ueber die Bildung von Gallenpigment aus Blutfarbstoff,' Pflüger's Archiv., Band IX, S. 53 und 329.

† 'On Haemoglobin Metabolism in Malarial Fever,' Roy. Soc. Proc., 1911, Vol. LXXXIII, p. 174.

In spite of the fact that in several of our experiments very large quantities (as much as 41 grammes) of haemoglobin were introduced into the circulation, we never observed any icteric condition of the skin or conjunctivae. On the other hand, the faeces were usually found to be semi-fluid and green after two or three injections of haemoglobin.

Owing to the fact that a certain amount of blood was withdrawn from the animal, and that this was replaced by haemoglobin solution, haemocrit records made during the experiments showed, as a rule, a progressive diminution of the volume of red blood cells as compared with that of the plasma. This decrease in the relative volume of the erythrocytes was, however, much more marked in the earlier experiments than in those in which the animal was kept on a dry diet and in which exceedingly strong solutions of haemoglobin were injected.

Condition of urine during experiment. The urine in the glass cannula was found to be tinged with haemoglobin five to ten minutes after the intravenous injection of the haemoglobin solution. Estimation of the percentage of haemoglobin passed in the urine showed that although a high degree of haemoglobinuria was quickly reached, nevertheless there existed no definite relationship betwen the concentration of haemoglobin in the urine and that present in the blood plasma, a very high percentage of haemoglobinuria being frequently observed after injection of comparatively small amounts of haemoglobin. As a rule, the maximum percentage of haemoglobinuria was not developed at once, but occurred some time (two to four hours) after the injection. A further point of interest is that after a second or third intravenous injection of haemoglobin the concentration of haemoglobin in the urine did not reach so high a level as that following upon the first injection, although in some instances the amount of haemoglobin circulating in the blood plasma was considerably higher.

It is clear that the percentage of haemoglobin present in the urine at any given time would of necessity depend upon several factors—firstly, the amount of free haemoglobin in the blood plasma; secondly, the rate at which it passed through the renal epithelium; and, thirdly, the amount of water which the kidneys secreted. When a comparison of the volume of urine passed and

the percentage of haemoglobinuria is made at regular intervals after the intravenous injection of haemoglobin solution, it is found that the latter varies to a certain extent inversely with the former, i.e., the more urine voided the lower the percentage of haemoglobin which it contains. Although the diuretic action of the sodium chloride solution is sufficient to account for the fact that the maximum degree of haemoglobinuria is not developed for some time after the injection, yet it affords no adequate explanation of why, after the second or third injection of haemoglobin, the percentage of haemoglobinuria is much lower than that following the first injection, even though in this case the blood plasma contained less free haemoglobin than in the former. It cannot depend upon the fact that the haemoglobin was diluted by a greater secretion of water, as, after the second or third injection, the amount of urine voided was distinctly less than earlier in the experiment. The only possible explanation is that either less haemoglobin was being excreted by the renal epithelium or that the haemoglobin was being held up in the renal tubules. This point will be returned to later in considering the microscopical appearances of the kidneys.

Although the first few specimens of urine examined after intravenous injection of haemoglobin solutions were perfectly clear without any deposit on centrifugalising, later specimens were turbid and contained a considerable quantity of solid material. Frequently, soft red masses of a gelatinous consistency closely resembling clots were passed. Microscopical examination of the deposit in the earlier stages showed that it consisted almost entirely of granular débris and casts. The clot-like masses themselves were composed of granular débris held together by mucoid material. The individual granules were at first of small size, although later in the experiment casts consisting of very large granules (3-4 μ) were frequently seen. They were of a brownish red colour, similar to that of red blood cells in urine, and were obviously derived from haemoglobin. In addition to this granular material there were also large flattened epithelial cells originating from some portion of the urinary tract below the kidneys.

Later, the character of the deposit changed considerably. In addition to the granular material large numbers of renal epithelial cells—isolated and also in the form of epithelial casts (Fig. 1)—

were found. Both the free cells and also those of the casts were frequently loaded with fine brown granules. Occasionally, a complete cast of the epithelium lining a portion of a renal tubule was seen in the form of an epithelial cylinder filled with granular material. On a few occasions red blood cells were found and, rarely, well marked red cell casts (Fig. 1).

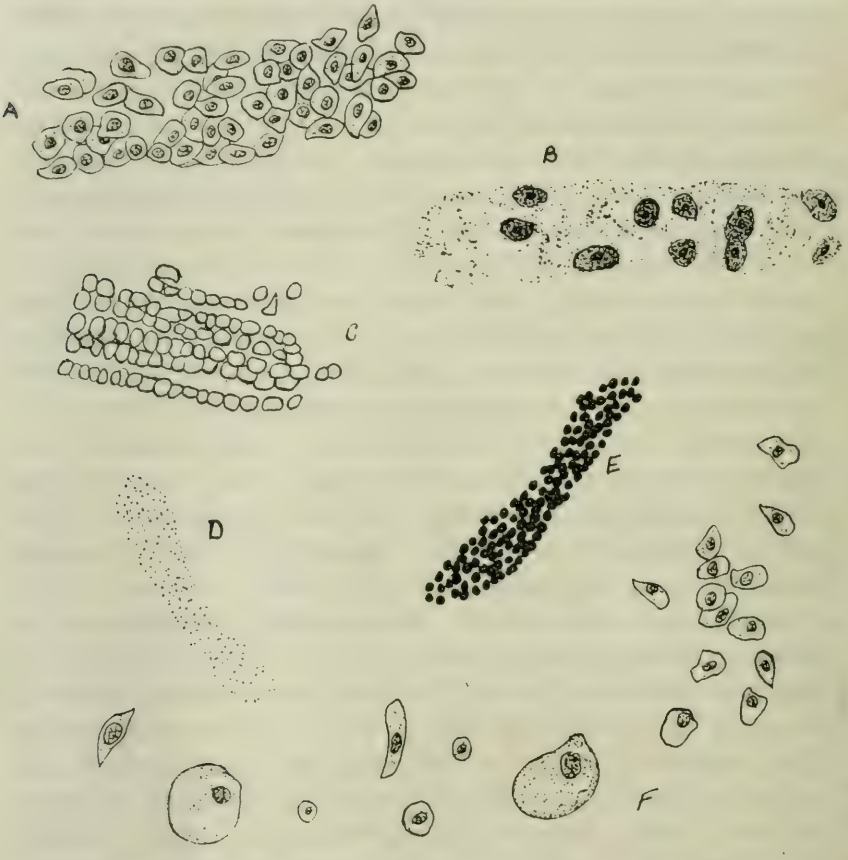


FIG. 1. Solid material passed in the urine of Rabbit 8 on the third day, during the period of almost complete suppression of urine. A—Epithelial cast. B—Finely granular cast in which are incorporated epithelial cells. C—Red blood cell cast. D—Finely granular cast. E—Cast consisting of coarse granules. F—Isolated epithelial cells. Magnification 300 diameters.

If no marked degree of suppression supervened, the urine cleared up after a certain time, depending upon the amount of haemoglobin injected, and the deposit of granular material and

epithelium gradually diminished, until, finally, the urine became normal. In several cases the flow of urine was greatly diminished or ceased altogether for a period. In Experiment 9 the animal developed complete anuria lasting over sixty hours; subsequently the secretion of urine gradually became re-established. In these cases (Rabbits 7, 8 and 9) the urine was greatly altered. No haemoglobinuria was observed. On acidifying with acetic acid and boiling, a dense white precipitate of albumen appeared. This was readily distinguishable from the dark chocolate precipitate which results when urine containing haemoglobin is boiled. On centrifugalisation there was a large deposit consisting of granular casts, some of which were built up of granules of very large size (3-4 μ), and enormous numbers of more or less degenerated renal epithelial cells and casts. This condition of the urine persisted until the animal's death.

Condition of the animal during the experiment.—Intravenous injection of large quantities of haemoglobin was in our experiments frequently followed by unfavourable symptoms. A considerable proportion of the rabbits injected died—some of them after even comparatively small doses (3·4 grammes). As a rule, the animal did not appear to be much affected during the first hour. Later, however, it became decidedly irritable, the slightest stimulus producing considerable reaction. Shortly before death the animal suddenly developed violent convulsions. Almost all the rabbits seemed to be more or less collapsed at first, but if they survived the injection longer than two hours they generally quickly recovered their normal condition. We were unable to discover the cause of death in those animals which died in convulsions. Careful post mortem examination conducted immediately upon the animal's death failed to reveal any signs of intravascular clotting either in the large veins, the heart, or in the pulmonary arteries.

Those animals which developed suppression of urine displayed characteristic symptoms. Chief among these were loss of appetite, rapid emaciation and considerable thirst (when once suppression of urine was established ordinary moist food was given). Death occurred in these animals two to eight days after the development of suppression of urine.

Condition of the kidneys. In considering the conditions

obtaining in the kidneys of these rabbits it is desirable to divide the experiments into two classes; firstly, those in which there was no indication of suppression of urine; and, secondly, those in which there was a more or less marked diminution in the amount of urine passed.

The kidneys of the first group varied considerably, both macroscopically and microscopically, according to the amount of haemoglobin solution injected and also according to the length of time which elapsed between the intravenous injection of

Table 2.—Weight of Kidneys in experimental and normal rabbits

No. of Experiment	Weight of rabbit, g.	No. of times haemoglobin solution was injected	Amount of haemoglobin solution injected g.	No. of hours animal lived after first injection of haemoglobin solution	Weight of Kidneys, g.		Remarks
					Right	Left	
I*	1,400	1	2·5	1·5	5·7	6·0	
II*	1,420	1	4·9	1·3	7·3	7·0	
[illegible]I	1,980	1	3·4	3·5	7·0	7·2	
III*	1,860	1	8·6	7	7·7	7·25	
IV*	1,570	2	11·3	22	6·8	6·5	
2	1,650	2	16·2	20	6·3	6·2	
3	1,750	4	29·4	38	6·7	6·8	
4	1,920	4	37·8	44	10·0	10·0	
5	2,870	5	16·3	98	10·5	10·5	
6	1,630	8	41·0	70	10·35	10·4	
7	1,235	7	23·7	68	11·55	10·75	Suppression of urine
8	1,740	3	28·2	94	11·0	10·75	
9	1,960	3	20·8	217	14·15	13·8	
Normal ...	1,550	—	—	—	6·0	6·1	
Normal ...	2,300	—	—	—	7·2	7·4	

haemoglobin and the removal of the kidneys. The kidneys of those animals which died after a single injection were but slightly altered in appearance. They were congested and of a dark brown or chocolate colour, but were not definitely enlarged (Table 2). Microscopically, a number of the convoluted tubules were seen to contain brownish coloured plugs. These were also present to a less extent in the tubules of the sub-cortical zone. There was no trace of solid material in the meniscus of Bowman's capsules nor in the collecting tubules of the papilla (ducts of Bellini). No dilatation of the tubules or glomeruli was observed.

* These experiments are not included in Tables 3-11.

A much more striking picture was afforded, however, by those kidneys which were removed after several injections of haemoglobin. Here these organs were distinctly enlarged and heavier than those of normal rabbits (Table 2). As before they were of a dark chocolate colour. On section the cortex was found to be thicker than normal, and the whole kidney was seen to be traversed by dark lines, giving it a markedly striated appearance. Frequently these dark coloured streaks were grouped in fan-shaped areas (Fig. 2), whilst the aspect of the intervening spaces was normal. The microscopical appearances presented by the kidneys were very characteristic. A large number of the convoluted tubules and tubes of Henle were filled with plugs of granular material, and here and there epithelial cells were found intermingled with the granular casts. The casts extended down into the large collecting tubules of the papilla. As before, no deposit was found in the meniscus of Bowman's capsules. Many of the convoluted tubules and tubes of Henle were distended and the epithelium in places had broken away from the basement membrane.

The kidneys of the animals belonging to the second group—those in which total or partial suppression of urine occurred—were profoundly changed. They were of a dark brown colour and greatly increased in size (Fig. 2) sometimes weighing more than twice as much as those of a normal rabbit (Table 2). On section they were found to be markedly oedematous. The cortical and sub-cortical zones were greatly thickened. Dark striations were visible, but they were not nearly so distinct as in the kidneys belonging to the previous group, and the appearance was confined chiefly to the sub-cortical portion of the pyramid. Microscopical examination showed that the whole structure of the kidney was radically altered. The tubules of the cortex and sub-cortical region were enormously dilated and many of them contained casts of granular material and epithelium in various stages of degeneration. The epithelium lining the tubules was in many places exceedingly thin, and in others had completely disappeared.

Scattered here and there between these large dilated tubules were small islands of tubules tightly packed together, so that their lumina were completely obliterated. Some of the glomeruli were enormously distended and others, like certain of the tubules, so

crushed as to be scarcely recognisable. The large collecting tubules were also much involved. In many places the epithelial cells had broken away from the basement membrane and were either lying

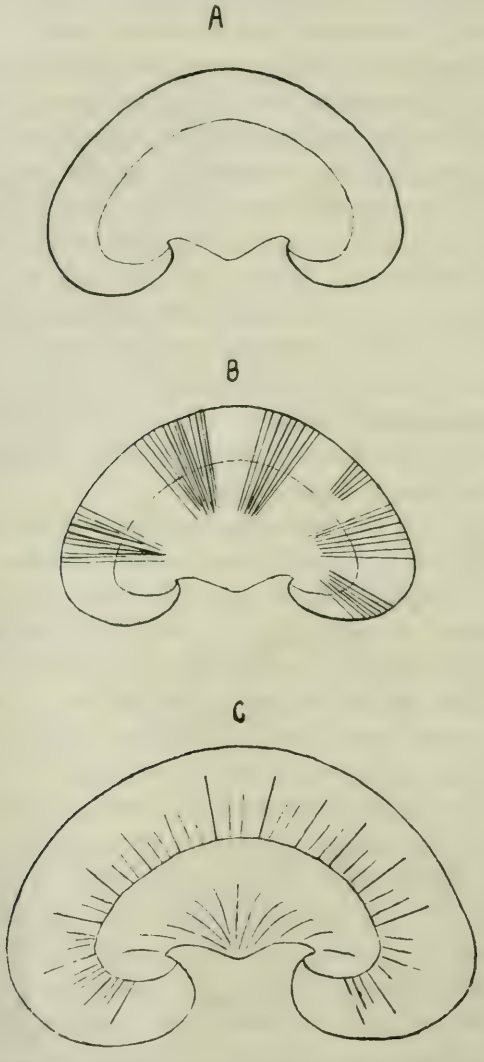


FIG. 2. Diagrammatic representation of the appearances found on section of—A, normal kidney; B, kidney of an animal which had received several injections of haemoglobin; C, kidney of Rabbit 9, in which there was complete anuria for 60 hours. Magnification 1·5 diameters.

loose within the tubule or had entirely disappeared. In other tubules the epithelium was intact, but the lumina were filled with

masses of epithelial cells and granular débris. Occasionally, a collecting tubule was found in which the lining epithelium was normal and the lumen entirely filled by a mass of granular material and isolated epithelial cells surrounded by a ring of epithelium evidently derived from a higher portion of the same collecting tubule.

As to the exact nature of the granular casts existing in the renal tubules and subsequently voided in the urine, it is difficult to speak with certainty. When examined in the fresh unstained condition their colour resembled closely that of the red blood cells. Moreover, they stain with various dyes (eosin, van Gieson, iron alum haematoxylin) in a manner precisely similar to the erythrocytes. There appears to be no doubt but that they are derived from haemoglobin, and we are of opinion that these casts are composed of minute droplets of haemoglobin (or of a very closely allied substance) held together by mucoid material. Of course this statement refers only to the casts existing in the renal tubules and urine examined within a few hours of the injection. The casts examined at a later period of time (four to eight days) after the haemoglobin injection, were obviously different. The material had in places changed to a light brown colour and no longer stained well. In other places it had become in part crystalline. We were unable to obtain the iron reaction with ammonium sulphide and ferrocyanide of potassium, probably because the decomposition of the haemoglobin had not progressed sufficiently far. This reaction was obtained by Werner in several cases of blackwater fever in man.

We have hitherto avoided any reference to the question as to which portion of the renal tubule is responsible for the elimination of haemoglobin. As we intend to discuss this question more fully in a future communication, we shall content ourselves here with stating that as a result of our observations we are of opinion that the haemoglobin does not pass through the glomeruli, but that it is excreted by the epithelium of the renal tubules, more especially by that of the convoluted tubules.

If the information obtained from examination of the urine be considered in conjunction with that derived from a study of the pathological condition obtaining in the kidneys, one is enabled to

form a fairly definite conception as to the effect which intravenous injection of haemoglobin has upon the renal secretion of an otherwise normal animal. The immediate result of such injections is a diuresis, dependent upon the action of the sodium chloride solution used for dissolving the haemoglobin. At the same time the renal epithelium commences to excrete haemoglobin into the tubules. At first this is quickly carried away by the rapid secretion of watery urine from the glomeruli. The result is a considerable flow of clear urine containing haemoglobin in solution. As the diuresis subsides the haemoglobin becomes deposited to a certain extent in the tubules. At this stage the urine is less in amount and contains a quantity of solid material consisting chiefly of granular casts and débris. Still later, especially when the degree of haemoglobinaemia is high, epithelial cells are shed from the lining membrane of the renal tubules. This is probably dependent upon one or both of the following factors. In the first place it is possible that the excretion of large quantities of a foreign substance like haemoglobin may be attended by injurious effects upon the renal epithelium. In the second place the presence of casts in the lumen of the tubules would tend to damage the lining epithelium mechanically, either by friction as the plug was forced down the tubule owing to the *vis a tergo* of the glomerular secretion or by causing a complete or partial block of the tubule, and, as a result, dilatation of that portion above the plug, and consequently separation of the epithelium from the basement membrane (Plate XIII, figs. 3 and 4). This stage represents the commencement of the development of suppression of urine. Gradually, as haemoglobin continues to be excreted by the renal epithelium more and more tubules become occluded. The situation in which complete blocking of the lumen first occurs appears to be, as a rule, the narrow tubules of Henle. Coincidently with the plugging of the tubules there is a marked diminution in the amount of urine voided and also of the haemoglobin it contains. The condition steadily progresses, until, finally, complete anuria (Case 9) results. After cessation of the flow of urine has lasted for a certain time a gradual re-establishment of urinary secretion occurs. The urine is loaded with albumen and is exceedingly turbid. On centrifugalisation a large deposit, sometimes equalling as much as

one-fourth of the volume of urine, is thrown down. This deposit consists of granular casts, epithelial casts, granular débris and degenerated epithelial cells. Urine of this nature continues to be passed until the animal's death. During the period of total or partial anuria marked changes occur in the kidneys. Owing to the continued secretion of the glomeruli those portions of the tubules above the plugs become enormously distended with fluid. As a result, the epithelium lining these portions becomes extremely thin and in places entirely disappears. The dilated tubules press upon adjacent tubules, so that on microscopic examination the latter appear of small size and their lumina are completely obliterated (Plate XII, fig. 1). After a time, owing to gradual disintegration of the casts plugging the tubes, the urine commences to escape into the ureter once more. The serum proteids and red blood cells pass through the damaged epithelium and those portions of the tubules devoid of epithelial cells, with the result that the urine is loaded with albumen. Although the casts may be to a great extent washed away in the course of the next few days, yet the renal tubules remain dilated and the serum proteids continue to escape into the urine until the animal's death, which in these cases is probably due to uraemia.

CONCLUSIONS

It is evident from these experiments that, under certain conditions, the mere passage of haemoglobin through the kidneys of a healthy animal is sufficient to cause suppression of urine owing to occlusion of the lumen of the renal tubules by plugs of granular material derived from the haemoglobin. The process is considerably facilitated by any factor which tends to lower the blood pressure of the animal, and as a result the secretion of water by the malpighian capsules. On the other hand, when the blood volume of the animal is kept up, as in Case 4, by the injection of saline solution (that of the haemoglobin solution which in this case was not particularly strong) and by feeding the animal on moist food, a large amount of haemoglobin (38 grammes) may be injected without any tendency to suppression of urine.

Table 3. Rabbit 1, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobin-aemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobin-uria	
1st ...	1 p.m.	1,980	22	...	...	38·0	...	...	
	2.35	...	...	3·4	...	...	...	...	
	2.45	...	...	...	6·5	...	...	...	
	2.55	...	...	...	...	...	4·4	3·4	
	3.15	...	...	...	...	...	5·5	2·1	
	3.30	...	...	...	...	...	4·5	1·7	
	3.45	...	...	...	...	...	5·0	1·0	
	4.45	...	...	...	...	...	4·7	4·0	
	5.25	...	...	...	...	...	1·0	3·9	
	5.45	...	...	...	...	...	1·25	3·6	
	6	...	...	...	1·4	...	1·2	4·0	Animal died with slight convulsions

Table 4. Rabbit 2, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobin-aemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobin-uria	
1st ...	9 p.m.	1,650	...	...	Trace	40·5	...	...	
	10	...	...	4·0	...	...	...	...	
	10.10	...	...	...	6·0	...	...	...	
2nd ...	12.15 a.m.	...	...	...	...	...	8·0	3·7	
	7	...	...	...	...	...	28·5	2·3	
	10	...	...	...	...	...	14·5	0·0	
	10.25	1,535	...	...	...	...	...	...	
	10.35	...	19	...	0·37	47·0	...	...	
	11.10	...	...	12·2	...	...	...	...	
	1 p.m.	...	...	...	...	...	10·0	4·6	
	3	...	...	...	...	...	4·5	15·7	
	5	...	...	...	5·4	34·0	12·0	7·0	
	5.25	...	15	...	...	...	...	...	
	5.45	...	...	...	...	...	...	...	Animal collapsed
	6	...	...	...	...	...	...	...	Death

TABLE 5. Rabbit 3, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobinaemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobinuria	
1st ...	11.10 a.m.	1,750	...	3·4	...	...	...	...	
	11.15	...	...	...	4·8	...	...	...	
	12.45 p.m.	...	...	...	...	...	2·6	2·5	
	3.30	...	...	...	...	...	8·0	5·1	
	5	...	18	...	0·34	43·5	5·0	0·5	
	5.30	...	...	9·6	13·0	...	...	...	
	7	...	...	...	...	...	10·0	3·2	
	10	...	...	...	...	...	11·5	7·2	
	11.30	...	16	...	2·4	35·5	5·5	3·4	
	12.25 a.m.	...	...	11·9	18·0*	...	...	...	* Calculated
2nd ...	1.20	...	...	...	...	...	19·0	3·7	
	7.40	...	...	...	...	...	27·5	5·5	
	10.30	...	...	...	...	...	16·0	2·0	
	11.45	...	11	...	1·8	30·0	...	...	
	12.10 p.m.	...	...	...	...	...	4·5	1·5	
	12.15	...	...	4·5	...	...	...	...	
	1	1,630	...	...	...	...	4·5	1·8	
	2.30	...	...	...	...	...	8·5	5·5	
	4.15	...	...	...	...	...	10·0	3·3	
	6.30	...	15	...	1·7	25·0	9·5	2·9	
	7.45	...	...	...	...	...	1·0	2·2	Animal died suddenly

TABLE 6. Rabbit 4, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobinaemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobinuria	
1st ...	6 p.m.	1,920	30	...	Trace	39·0	...	...	
2nd ...	11 a.m.	...	...	...	...	...	17·0	0·0	
	3.30	...	...	...	...	...	10·0	0·0	
	4.20	...	...	9·2	...	...	...	...	
	4.40	...	...	...	10·0	...	3·0	2·7	
	5.5	...	...	...	...	...	3·8	5·1	
	6.20	...	...	...	...	...	4·5	17·8	
	8.40	...	...	...	...	...	3·4	16·6	
	10.20	...	...	...	...	...	0·6	10·0	
	11.45	...	...	...	...	...	7·0	4·6	
3rd ...	12.5 a.m.	...	18	...	1·2	24·5	...	...	
	12.20	...	...	7·6	...	...	...	...	
	12.25	...	...	...	9·5	...	0·0	...	
	4	...	...	...	...	...	2·0	13·5	
	11	...	...	...	...	...	10·0	4·0	
	4 p.m.	...	...	...	...	...	3·5	0·0	
	4.30	...	...	...	...	...	2·0	0·0	
	4.50	...	22	...	0·3	23·7	...	...	
	5.40	...	...	13·0	...	...	...	...	
	6.50	...	...	...	...	...	12·0	4·8	
	10	...	...	...	...	...	...	...	
	10.45	...	...	...	...	...	18·0	7·3	
4th ...	8 a.m.	...	...	...	...	...	2·0	12·1	
					...	...	20·0	7·3	
	11.20	...	...	...	...	...	7·0	1·3	
	12.10	1,640	...	8·0	...	...	3·0	0·2	Animal died immediately after the injection

TABLE 7. Rabbit 5, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobin-aemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobin-uria	
1st ...	11.30 a.m.	2,870	26	...	...	33·0	...	...	
	3 p.m.	...	...	3·65	...	...	...	...	
	3.10	...	...	...	4·2	...	...	...	
	3.45	...	...	...	...	...	0·75	2·7	
	4	...	...	...	2·9	...	...	...	
	4.45	...	...	...	...	...	2·6	6·8	
	5.45	...	...	...	...	...	3·8	5·1	
	6.10	...	...	...	...	...	1·8	2·5	
	6.45	...	...	...	...	...	3·0	3·0	
	8	...	...	...	...	...	5·0	0·45	
	8.15	...	...	...	0·4	30·0	...	...	
	8.45	...	30	...	...	...	...	...	
	10.45	...	...	3·4	...	...	...	...	
	11	...	...	...	4·5	...	...	...	
2nd ...	10 a.m.	...	...	...	...	...	11·0	2·0	
	10.30	...	...	...	...	...	1·0	0·2	
	11	...	...	...	0·3	26·4	...	...	
	11.30	...	37	...	...	...	...	...	
	1.45 p.m.	...	...	4·0	..	...	...	...	
	1.50	...	...	...	5·0*	....	...	...	* Calculated
	5.30	...	...	...	...	...	3·0	5·8	
	6	...	...	...	...	...	1·0	1·5	
	10.45	...	34	...	0·15	21·0	13·0	0·2	
3rd ...	12.10 a.m.	...	...	3·5	...	...	...	...	
	12.20	...	...	...	4·5*	...	...	...	* Calculated
	9	...	...	...	...	...	38·0	0·5	
	11	...	...	...	...	...	10·0	0·0	
	11.15	...	25	...	...	14·0	...	...	
	1.45 p.m.	...	...	1·8	...	...	...	...	
	1.50	...	...	...	1·5	...	...	...	
	2.15	...	...	...	...	...	3·0	1·5	
	4	...	...	...	...	...	2·7	6·4	
	5	...	...	...	...	...	4·8	1·5	
	11.30	...	...	...	...	...	17·0	0·2	
4th ...	10 a.m.	...	...	...	...	...	33·0	0·0	
	11 p.m.	...	...	...	...	...	24·0	0·0	
5th ...	10 a.m.	...	...	...	...	...	20·0	0·0	
	5 p.m.	...	...	...	...	...	7·0	0·0	Animal died

TABLE 8. Rabbit 6, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobinaemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobinuria	
1st ...	12.20 p.m.	1,630	18	...	Trace	42·5	...	...	
	1.18	...	...	3·0	...	...	...	...	
	1.23	...	...	...	4·7	...	...	...	
	2.50	...	...	...	...	...	1·9	6·3	
	4	...	...	...	...	...	1·8	10·33	
	5	...	...	...	...	...	1·3	17·9	
	5.45	...	...	...	...	...	1·4	5·0	
	6.10	...	...	4·4	...	...	2·2	3·0	
	6.14	...	...	...	5·8	...	...	...	
	9	...	...	...	...	...	2·0	7·2	
	11.30	...	16	...	1·3	33·0	2·5	21·0	
2nd ...	12.5 a.m.	...	...	4·7	...	...	...	...	
	12.15	...	...	...	7·0*	...	...	...	* Calculated
	10	...	...	...	...	...	7·0	8·3	
	11.50	1,580	...	...	...	...	2·5	1·5	
	12.5 p.m.	...	...	6·0	...	...	...	...	
	12.10	...	...	...	9·0	...	...	...	
	3	...	...	...	...	...	4·5	4·8	
	4.30	...	...	...	...	...	2·0	4·8	
	6.30	...	...	...	...	...	3·7	3·45	
	8.55	...	11	...	1·5	29·6	...	...	
	10	...	...	5·4	...	...	...	...	
	10.15	...	...	...	9·0*	...	...	...	* Calculated
3rd ...	12.5	...	...	...	...	...	2·5	3·65	
	7.10	...	...	...	...	...	10·0	2·2	
	10.15	...	...	...	...	...	4·75	0·7	
	10.25	...	20	...	1·2	22·0	...	...	
	11	...	...	6	...	...	...	...	
	11.5	...	...	...	9·5*	...	...	...	* Calculated
	1 p.m.	...	...	...	...	...	1·5	2·7	
	3	...	...	...	...	...	4·0	5·0	
	5.30	...	...	...	...	...	3·75	4·25	
	5.45	...	...	7	...	...	...	...	
	5.50	...	...	...	10·5	...	...	...	
	8.25	1,540	...	...	...	...	...	...	
	8.30	...	...	...	...	...	1·7	3·7	
	11.30	...	12	...	5·45	15·5	2·8	3·18	
	11.50	...	...	4·45	...	...	...	...	
4th ...	12.50 a.m.	...	...	...	...	...	0·0	...	
	7.10	...	...	...	...	...	0·8	3·0	
	10.30	...	...	...	...	...	0·7	2·4	Animal died

TABLE 9. Rabbit 7, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobinaemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobinuria	
1st ...	3.45 p.m.	1,235	...	1·5	...	...	...	...	
	4.30	...	...	...	...	...	3·0	4·2	
	5.45	...	14	...	1·3	35·0	2·0	2·3	
	5.54	...	...	3·0	...	...	...	...	
	6.30	...	...	...	...	...	5·0	4·0	
	7	...	...	...	...	...	1·0	8·0	
	9.30	...	18	...	1·0	23·75	2·0	2·0	
	10.20	...	...	4·6	...	...	...	...	
2nd ...	1.15 a.m.	...	...	...	...	...	7·0	2·7	
	1.35	...	...	3·9	...	...	...	...	
	7.15	...	...	...	...	...	16·5	1·9	
	10.30	...	...	...	...	...	9·0	1·6	
	1.30 p.m.	1,100	...	...	...	...	...	...	
	2.40	...	...	3·0	...	...	0·5	2·2	
	7	...	...	...	...	...	0·75	1·8	
	9	...	...	...	...	...	0·25	1·8	
	11.30	...	...	...	...	...	0·8	3·6	
	11.35	...	...	3·3	...	...	...	...	
3rd ...	12.5 a.m.	...	...	...	...	...	0·0	...	
	1.30	...	...	...	...	...	0·3	4·0	
	7.30	...	...	...	...	...	0·5	1·5	
	11.30	...	...	...	...	...	0·5	1·5	
	12.5 p.m.	1,120	...	...	...	...	...	...	
	12.40	...	...	4·4	...	...	...	...	
	5	...	...	..	...	...	·9	2·0	
	11	...	...	...	...	...	1·5	0·9	
4th ...	12.5 a.m.	...	...	...	...	...	0·0	...	
	9	...	...	...	...	...	3·2	0·4	
	11	...	...	...	...	...	...	...	Animal died

TABLE 10. Rabbit 8, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobinaemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobinuria	
1st ...	10 p.m.	1,740	...	...	...	...	...	...	
	10.45	...	...	4·3	...	...	...	...	
2nd ...	7 a.m.	...	...	...	...	...	32·0	1·2	
	10.30	...	...	...	...	...	18·0	0·2	
	10.35	1,630	...	...	...	...	...	...	
	10.45	...	20	...	0·4	47·5	...	...	
	11.55	...	...	12·9	...	...	...	...	
	12.5 p.m.	...	...	...	16·9	...	...	...	
	1	...	...	...	...	...	8·0	3.1	
	3	...	...	...	...	...	5·0	5·0	
	5	...	...	...	...	...	16·0	6·7	
	7	...	...	...	...	...	2·0	8·9	
	9.15	...	...	...	...	...	3·0	5·0	
	10.5	...	...	...	...	...	0·6	4·1	
	11.35	...	...	...	...	...	1·4	3·0	
	11.50	...	...	...	2·2	42·0	...	...	
3rd ...	12.45 a.m.	...	...	11	...	...	...	...	
	12.50	...	...	...	15·5*	...	...	...	* Calculated
	2	...	...	...	...	...	0·0	...	
	7	...	...	...	...	...	3·4	4·8	
	12.5 p.m.	1,600	...	...	...	...	0·7	5·0	
	12.50	...	...	...	...	...	0·7	4·6	
	1	...	...	...	...	...	0·0	...	
	11	...	...	...	...	...	0·9	2·9	
4th ...	1 a.m.	...	...	...	...	...	0·0	...	
	9	...	...	...	...	...	4·0	0·75	
	1 p.m.	1,575	...	...	0·3	45·0	1·3	0·0	Heavy white precipitate of albumen on acidifying with acetic acid and boiling. Considerable deposit of granular casts and epithelial cells
	6	...	...	...	...	...	2·2	0·0	
	11.55	...	...	...	...	...	2·0	0·0	
5th ...	1 a.m.	...	...	...	...	...	0·0	...	
	3	...	...	...	...	...	0·8	0·0	
	7	...	...	...	...	...	0·9	0·0	
	8	...	...	...	...	...	0·0	...	
	8.30	1,500	...	...	...	...	0·0	...	Animal died

Table 11. Rabbit 9, injected intravenously with haemoglobin solution.

Day	Time	Weight g.	Amount of blood withdrawn c.cm.	Amount of haemoglobin injected g.	Examination of Blood		Examination of Urine		Remarks
					Percentage of haemoglobin-aemia	Haemocrit	Amount of urine passed c.cm.	Percentage of haemoglobin-uria	
	10 a.m.	1,960	...	...	...	...	...	...	
1st ...	11	...	30	...	Trace	29·5	...	...	
	3 p.m.	...	...	10·8	...	...	...	...	
	3·5	...	...	...	12·6	...	...	...	
	4.15	...	...	...	...	...	2·0	8·0	
	5	...	...	...	...	...	1·7	11·4	
	5.15	...	...	...	...	...	2·7	9·75	
	8	...	...	...	...	...	11·0	4·5	
	9	...	16	...	2·8	20·4	9·0	3·0	
	10.30	...	...	...	...	...	1·5	1·6	
2nd ...	12.35 a.m.	...	...	7·5	...	...	...	...	
	12.40	...	...	...	9·7	15·5	0·9	3·2	
	1.30	...	...	...	...	...	0·0	...	
	7.30	...	...	...	...	...	7·1	1·1	
	10.30	1,850	...	...	...	...	0·0	...	
	1 p.m.	...	...	...	...	...	0·0	...	
	4	...	...	...	5·25	...	0·0	...	
	8.45	...	...	2·5	...	...	...	...	
	9.15	...	...	...	...	...	0·0	...	
	11.30	...	...	...	...	...	0·0	...	
3rd ...	10 a.m.	1,850	...	...	...	...	0·0	...	
	11.30	...	...	...	...	...	0·0		
	5 p.m.	...	...	...	...	18·5	0·0	...	
	11.30	...	...	...	...	...	0·0	...	
4th ...	9 a.m.	1,840	...	...	...	...	0·0	...	
	1 p.m.	...	...	...	...	...	0·6	0·0	
	3	...	...	...	Trace	18·5	0·0	...	
	5	...	...	...	...	...	1·5	0·0	
	10	...	...	...	...	...	1·0	0·0	
5th ...	8 a.m.	...	...	...	...	...	2·0	0·0	
	1 p.m.	...	...	...	...	...	1·6	0·0	
	10	...	...	...	...	...	7·3	0·0	Heavy white precipitate of albumen on acidifying with acetic acid and boiling
6th ...	12.5 a.m.	...	...	...	...	...	1·4	0·0	
	8	...	...	...	...	...	28·0	0·0	
	10	1,750	...	...	...	...	5·0	0·0	
	3 p.m.	...	...	...	...	...	2·0	0·0	
	6.30	...	...	...	...	...	2·5	0·0	
7th ...	12.5 a.m.	...	...	...	...	...	5·0	0·0	Considerable deposit of granular casts and renal epithelial cells
	10	1,640	...	...	...	...	12·0	0·0	
	7 p.m.	...	...	...	...	...	5·0	0·0	
	11.3	...	...	...	...	...	4·0	0·0	
8th ...	10 a.m.	...	...	...	...	...	13·0	0·0	
	5 p.m.	...	...	...	...	...	6·0	0·0	
9th ...	10 a.m.	1,460	...	...	...	...	10·0	0·0	
	5 p.m.	...	...	...	...	...	10·0	0·0	
10th ...	10 a.m.	1,390	...	...	...	...	2·0	0·0	
	4 p.m.	...	...	...	...	...	0·0	0·0	Animal died with slight convulsions

DESCRIPTION OF PLATES

The sections were stained by Heidenhain's iron alum haematoxylin method and the figures drawn with an Abbé camera lucida.

Plate XII

Fig. 1.—Section of renal cortex of Rabbit 9. Many of the convoluted tubules are greatly distended and in some the lining epithelium is considerably flattened. Scattered between the dilated tubes are islands of tubules tightly packed together, so that their lumina are completely obliterated. Two Bowman's capsules are seen in the section. A number of the dilated tubules contain plugs of granular material. Magnification 240 diameters.

Fig. 2.—Transverse section of renal pyramid (sub-cortical portion) of Rabbit 6, showing many dilated tubules (tubes of Henle and collecting tubules) completely plugged with granular material. Magnification 350 diameters.

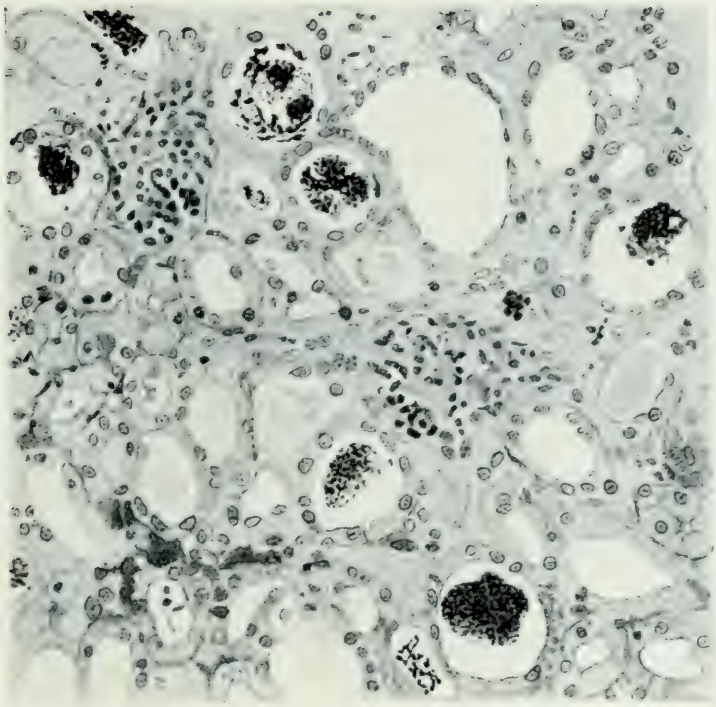


FIG. 1.

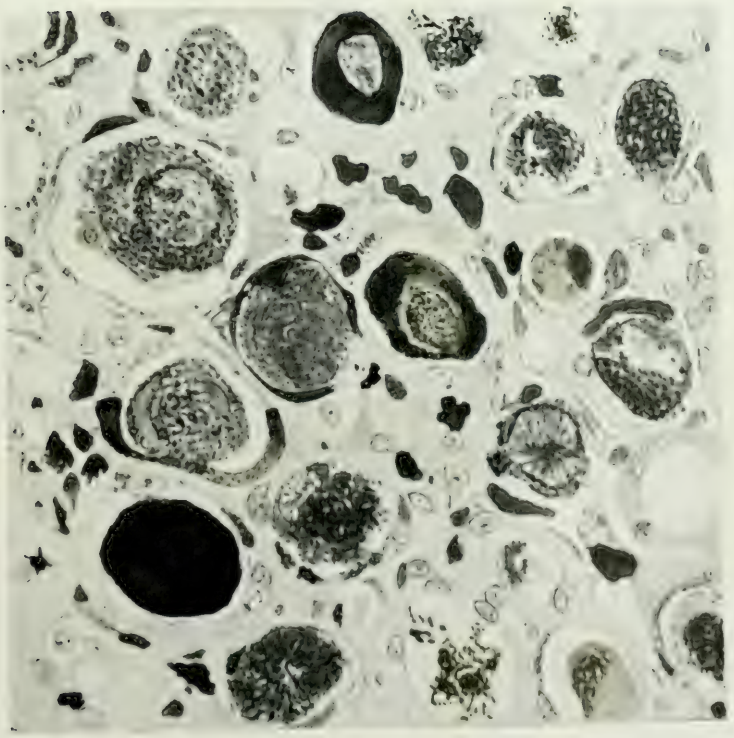


FIG. 2.

A. M. B., del.

P. J. Press, Imp.

ON THE RELATION OF THE ORGANIC PHOSPHORUS CONTENT OF VARIOUS DIETS TO DISEASES OF NUTRITION, PARTICULARLY BERI-BERI

BY

G. C. E. SIMPSON, B.A., M.B., B.Sc.,

AND

E. S. EDIE, M.A., B.Sc.

PART I.

(*From the Laboratories of Tropical Medicine, and of Bio-Chemistry, Liverpool University.*)

(*Received for publication* 10 *June*, 1911)

In November, 1910, Professor Ross called our attention to Fraser and Stanton's work on The Etiology of Beri-beri (1910), and asked us to enquire into the subject with a view to further researches on similar lines and at the same time supplied us with a large sample of a rice, the use of which as a diet had been associated with an outbreak of Beri-beri.

Shortly after we had commenced our work the monograph of Schaumann (1910) appeared in the Archives for Ship- and Tropical-Hygiene, and this work was found to be so complete and exhaustive that further investigation of the main facts seemed superfluous, and we turned our attention to confirming some of the newer facts and to further investigations of points—mainly of a chemical nature—arising out of his results.

Schaumann's work is so important that it seems advisable to give here an abstract of the more important sections of his monograph, which should be consulted for full details and also for the exhaustive bibliography.

After a historical review of the previous theories of the causation of Beri-beri and of the work of himself and others refuting these conceptions—e.g., a specific infection, contamination from without

by toxic bodies, or by autogenous development of such bodies in the diet, etc., he proceeds to the consideration of the work which connects 'Tropical Beri-beri' with the use of highly prepared rice—e.g., rice in which the husk and outer layers of the grain have been removed by the process of milling—and of that which connects 'Ship Beri-beri' with the use of similar rice as the staple diet, or with the use of preserved foods and other restricted or unsuitable diets.

Braddon (1909) first called attention, in 1901, to the fact that Beri-beri was prevalent in those Eastern tribes and races, e.g., Chinese, who used a highly-milled rice as their staple diet, but did not occur among races such as the Tamils, who cured their rice in such a way that the pericarp was not removed from the grain in the process of milling; Fletcher (1907) confirmed these observations, showing that the substitution of whole rice for polished rice in the dietary of the inmates of an asylum abolished Beri-beri, while the subsequent reversing of the diet led to an outbreak of the disease. Since 1907 polished rice has been abolished finally from the dietary of the inmates and Beri-beri has ceased, though previously extremely prevalent and causing many deaths. Fraser (1909) obtained similar results with gangs of labourers inhabiting separate compounds, and others have added further confirmation. The practical application of these observations by hygienists in our Eastern Possessions, in the Japanese navy and army, etc., has resulted in a great diminution of the incidence of the disease.

Turning now to another side of the question, Schaumann refers to the experimental production of polyneuritis in fowls; Eijkman (1897), in Batavia in 1896, discovered that fowls fed entirely on any variety of polished rice became lame and ultimately died, and demonstrated lesions in the peripheral nerves and anterior horn cells of the dead animals. On whole rice, however, no such symptoms were developed, and addition of the polishings to the prepared rice prevented their onset.

He further proved a similar difference between barley and prepared barley, and finally discovered that rice, barley and rye are no longer adequate after heating for a time to a temperature of 120° C., and birds fed on the above fall ill and die with typical appearances of peripheral neuritis. For diets so treated the name 'denaturised' is used.

Grijns (1908), in Batavia and Holland, confirmed Eijkman's results, and showed the development of polyneuritis in fowls when polished rice, sago and tapioca formed the sole diet, and that the same train of symptoms arose on a diet of flesh which had been heated to 120° C.

He discovered that the addition of small quantities of 'Katjang-idjo' beans (*Phaseolus radiatus* L.) rendered safe a diet previously injurious, and these beans also lost their preventive power if heated to 120° and *further demonstrated that the power of a rice to produce Beri-beri varied directly with the degree of preparation, that is with the extent to which the pericarp (or silver skin) was removed in milling.*

Axel Holst and Frölich (1907), working at Christiania with special reference to Ship Beri-beri and to scurvy, carried out a series of researches on pigeons and guinea-pigs, using various limited diets. Their results in the main confirm those of Eijkman and Grijns, both for prepared rice and barley and for heated foodstuffs. They demonstrated, in addition to the polyneuritis, marked emaciation in the birds and animals used, and also oedema of the subcutaneous tissues and muscles; further in mammals they found changes in the gums accompanied by loosening of the teeth.

In addition they examined various sorts of bread, and found that wheat bread was harmful both to pigeons and to guinea-pigs—bread baked with yeast, however, was much less harmful than bread baked with baking-powder. It should be noted that guinea-pigs are much less resistant than fowls to such restricted diets and that restriction of these animals to one sort of grain, whether ground or not, is invariably deleterious; rarefaction of the bones, haemorrhages in the neighbourhood of the epiphysial line, degenerations of bone marrow, and sponginess of the gums are often produced.

Holst further determined the amounts of peas, unshelled barley, etc., which required to be added to diets in order to prevent the onset of symptoms, and also calls attention to the fact that preserved meat has often been denaturised by heating to 120°.

Fraser and Stanton (1910) further confirmed these observations with regard to the association of polished rice and polyneuritis.

Schaumann (1908) and Fraser and Stanton (1909) (independently) discovered that diets which produced peripheral neuritis

were invariably poor in phosphorus, and that substances (e.g., rice meal, Katjang-idjo), which have the power of preventing the development of neuritis, are, on the contrary, rich in that substance; *indeed that the smaller the percentage of a diet in phosphorus the greater is its influence in producing Beri-beri in man, or neuritis in fowls.*

Schaumann thought that the active principle containing phosphorus was probably nucleic acid, and Grijns, following up this hypothesis, experimented with the nucleo-proteins of Katjang-idjo; no curative effect was observed with nucleins extracted by alkali, but some slight postponement of death was observed in neuritic birds treated with the phosphorus-containing extract obtained with hot water.

Schaumann further gives exhaustive tables of the composition of various food stuffs—grains (fresh and prepared), peas, beans, potatoes, meat, etc., both in the fresh and dried states, showing their composition with reference to protein, carbohydrate and fat, and in particular their various phosphorus-containing compounds—inorganic, phytin-like compounds, nucleins and phosphatides—and discusses the present state of our knowledge of the metabolism of these bodies.

Schaumann next proceeds to a critical review of the various theories of the etiology and points out that neither place, climate, nor season appear to be of importance, and that no specific organism has been isolated though many bacteriologists, and even Koch himself, have made careful investigations; Fletcher and Fraser also carefully excluded the question of infection since they showed that whether Beri-beri was present or absent among the inmates of a building or compound it could be banished by feeding with partly milled rice or called forth by feeding on wholly milled rice.

Braddon, with respect to Beri-beri, and Eijkman, with reference to Polyneuritis in birds, at first assumed that some toxic agent was present in the kernel of the grain which gave rise to Beri-beri and that the antidote to this toxin was present in the pericarp, and that so no bad effect followed feeding with the whole grain; but, if the pericarp were removed, the toxin already present or elaborated during digestion was free to exercise its effects. Others assumed that the fully shelled rice became contaminated more easily by toxic bodies (e.g., arsenic) and so caused ill effects, but Fraser and Stanton showed

that polished rice was equally deleterious when freshly ground as when it had long been exposed to the risk of contamination.

In spite of careful chemical investigations it has proved impossible to isolate any toxic body from polished rice, from denaturised (heated to 120°) flesh, or from other substances which cause Beri-beri or Polyneuritis; Fraser and Stanton further showed that sera, flesh, and other products of neuritic fowls caused no deleterious symptoms when administered orally or by injection to sound animals.

Schaumann then considers the theories which ascribe the development of Beri-beri to a faulty nutrition, and he calls attention to the theories of Nocht (1908); the latter ascribes both the multiform manifestations of Tropical Beri-beri and the usually milder Ship Beri-beri (which appears to be somewhat allied to scurvy), to dietetic errors; and lays special stress on the fact that it is not a question of defect of the main components of food stuffs—proteid carbohydrate and fat—but of some subtle defect of the less known constituents—enzymes, complements, compound proteids, etc.

Schaumann himself, however, is inclined to consider the substance or substances of importance to be organic compounds of phosphorus. He resolved first of all to investigate the possibility of the neuritis being due (1) to the development of oxalic acid by fermentation or to acidosis from deficiency of alkaline salts in the grain; (2) to the removal of ferments or other thermolabile substances by milling or 'denaturisation,' and (3) to investigation of the influence of phosphorus compounds, organic and inorganic.

He points out that the exact chemical nature of the phosphorus compounds in food stuffs and the body is but imperfectly known, that the methods of separation are but approximate and uncertain in many cases, and that the isolated products are probably modified by the processes of extraction, and so differ in physiological effect from their precursors.

Subject to modifications necessitated by the light gained in the process, he resolved to try diets, as polished rice and denaturised foods, which were known to produce neuritis, etc., and see whether any favourable results are obtained by the administration of such diets, with the addition of:—

1. Known organic and inorganic compounds of phosphorus.
2. Small amounts of substances rich in phosphorus, e.g., rice

meal, yeast, Katjang-idjo, wheat meal, testicular extract, or extracts from roe, etc.

3. Phosphorus compounds isolated by him from articles of food, e.g., yeast, Katjang-idjo, etc.

Special stress was attached to investigations with denaturised foods in which the defect was probably simpler than in milled foods, and Schaumann also planned to investigate microscopically and chemically the nerves and other tissues of the animals experimented on, and to carry out chemical investigations on fresh and preserved foods—foods from Beri-beri ships (and moulds, etc., contaminating them)—and also on the phosphorus excretion in faeces and urine of birds and Beri-beri cases.

Schaumann's first results show that excess of oxalic acid (or other toxic products) and deficiency in autolytic enzymes can play no part in the etiology of Beri-beri nor of Polyneuritis in fowls, and further that the fault of diet lies neither in deficiency of proteins nor of inorganic salts, since addition of these substances to a diet which gives rise to Polyneuritis has no protective influence, and also since the diets which Fletcher, Fraser and Ellis found to cause Beri-beri contain respectively 92, 103 and 99 grams of protein per man per day, a figure quite sufficient to maintain equilibrium, especially considering that Fletcher and Ellis were dealing with inmates of institutions for mental disorders and not with men engaged in hard manual labour. Turning now to phosphorus compounds, Schaumann gives exhaustive details of the various organic and inorganic compounds present in foodstuffs, and in the various organs of the body with their characteristics and the methods of extraction, etc. He then passes to a consideration of phosphorus metabolism; an adult man at work requires about two grams of phosphorus daily on mixed diet, while a dog seems to require less than 0·5 gram per day; birds in some instances require much larger amounts than mammals of the same size.

Passing on, he considers the metabolism of each of the better known classes of organic phosphorus compounds, phosphatides, nucleoproteins, phytin, etc., and also inorganic phosphates, giving an account of their absorption, assimilation, retention and excretion.

He calls attention to the fact that phosphorus is present in especially large amounts in the organs whose functions are most

complicated and important; further, the main organic phosphorus compounds are in large part assimilated by the alimentary tract as organic compounds, without the previous splitting off of the phosphoric acid, and the organs of man and the higher animals have further a marked power of storing excess of such compounds and of drawing on this store at times when these are deficient in the diet. He quotes Miescher's well-known work on the increase of salmon roe at the expense of the phosphorus of its muscles, and suggests that pregnant females may store up phosphorus to be subsequently given out in the milk. In addition to this, new-born animals usually possess a store of phosphorus proportionate to the length of time which will pass before they can forage for themselves (as is known to be the case also with iron). In times of hunger the animal organism is more economical of its phosphorus than of any other inorganic constituent, and seems to have a more urgent need of its phosphorus than even of its proteids.

Albu and Neuberg (1906) consider that it has been practically demonstrated that the body has not the power to synthesise the organic phosphorus compounds necessary for the life of the cells from phosphorus-free proteins and inorganic phosphates, *but that animal life is dependent for these substances (as for proteins and carbohydrates) on the synthetic powers of the vegetable kingdom.*

Schaumann next resolves to attempt as far as possible experimentally to determine the way in which an absence or deficiency of each or all of these compounds in the diet influences the animals, as Liebig has worked out the influence of salts on the growth of plants. The problem is much more difficult in the animal kingdom, however, since the substances are of great complexity, present great variety in each class, and are not only difficult to isolate but undergo important modifications in the processes of separation. These difficulties may lead to failure or to the results obtained experimentally being wrongly interpreted.

All earlier observers of neuritis, etc., in animals had considered that the causation of the lesion was to be attributed to some poisons (toxin, oxalic or other acid), which developed endogenously or exogenously in the nutriments producing them, and which could be neutralised by certain (equally hypothetical) anti-bodies which are present in certain food-stuffs, e.g., rice meal, *Phaseolus radiatus*,

etc. Fraser and Stanton (1909) have called attention to a parallelism between the lack of phosphorus in the food and the onset of Beri-beri in men and of neuritis in cattle, and in that have followed the suggestion made by Schaumann in 1908.

Schaumann's earlier experiments were not strictly conclusive as showing that lack of organically combined phosphorus was the most important and indeed the only factor in the etiology of Beri-beri, and his further experiments are planned with a view to attempting to find such conclusive proof.

Researches on Pigeons. Polished rice (20 grams per bird per day) forms, in the majority of cases, the basis of the food. It is used in the form of a pap or porridge, to which various amounts of the different substances can be added, with a view to determining their protective power; in other cases barley or barley bread was used as a basis, but these experiments have to be viewed independently of the rice experiments if the average time required for the development of neuritis, etc., is to be taken as a measure of the possession or lack of protective power.

Well-grown and well-nourished pigeons are found to be the most suitable for experiment, young animals and fancy breeds being less serviceable. Birds are specially suitable because their metabolism is very regular, and deficiency in the food is rapidly indicated. Further, the curative influence of protecting substances on sick birds is much more rapidly noticeable than on mammals.

Control experiments with pigeons showed that death occurred in the average period of thirty-five days on polished rice or rice bread; the birds exhibiting more or less severe appearances of lameness and losing 41 per cent. of their original weight.

If the rice is previously thoroughly extracted with cold water, the birds die much faster, in an average period of twelve days; extraction removed only 3·43 per cent. of the original proteid but 36 per cent. of the original phosphate; a difference of proteid per bird per day which would be unlikely to so hasten the end.

Non-protective Substances. Addition of dried egg-albumen, of albumen metaphosphate, calcium glycero-phosphate, and of inorganic salts (with or without phosphates), had no marked influence in either direction, as will be seen from Table I.

Nucleic acid from yeast prepared for commercial purposes

(scientific and medicinal) appeared to have a very unfavourable influence, but a specially manufactured sodium nucleate, carefully prepared to break up the molecule but little, seemed to have a slightly favourable influence. The birds only lived a few days longer than on rice alone, certainly, but the lameness was not marked.

Protective Substances. The meal or bran from the outer parts of grain which is removed in milling, however, had a very different influence when added to the polished rice pap used for feeding.

With two grams of rice meal (P_2O_5 3·8 per cent.) added to the pap for the day's ration, the birds remained fit and strong (for seventy-two days), and indeed gained in weight—1·5 gram per bird per day scarcely sufficed to keep them strong, and with 1 gram loss of weight commenced.

Equally favourable was the influence of the addition of wheaten bran, which contains 1·1 per cent. of P_2O_5. Four birds each received 2·5 grams of this per day, with the usual ration of rice pap. They lost some 20 per cent. of their weight, though otherwise remaining well for twenty-eight days; the ration was raised to 5 grams each per day, but as the birds did not recover their initial weight it was raised fourteen days later to 7·5 grams, and the birds in the next twenty-five days not only reached their original weight, but actually added a further 10 per cent., and were perfectly fit and active.

In a further set of five pigeons, two grams per bird per day of dried brewer's yeast (P_2O_5 4·2 per cent.) was added to the diet of rice pap; the birds remained well, but in two months lost a little (3·5 per cent.) of their weight. The allowance of yeast was reduced to one gram per bird daily, and except for the loss of a further 3·5 per cent. of their weight, they were at the end of a month apparently just as well as at the beginning of the experiment three months earlier.

Schaumann now compares these three 'protecting' substances as regards the phosphorus and proteid contents of the daily allowance needed to keep a pigeon well and maintain its weight, and it will be noticed that the phosphorus contents almost coincide.

Substance	Daily allowance (gram)	P_2O_5 content (gram)	Protein content (gram)
Yeast	1·5	0·063	0·55
Wheat bran ...	5·0	0·055	0·72
Rice meal ...	1·5	0·057	0·16

Curative Effects. In addition to trying the protective influence of these various substances when added to the polished rice diet from the beginning of the experiments, Schaumann also tried their power of curing pigeons which were already suffering from neuritis and in many cases were apparently very near to death.

In strange contrast to the fact that it exercised no protective power when administered in the diet from the beginning, is the effect of nucleic acid prepared from yeast when administered to birds already lamed by neuritis. Of fourteen cases in which this was administered (forced feeding), six died before the nucleic acid had passed out of the crop; in five cases with repeated forced feeding with nucleic acid and rice, some improvement manifested itself, but was only transitory, and the birds died in five days—the lameness which at first lessened having again returned.

In the remaining three cases the result was even better; rice pap containing 3·5 per cent. of yeast nuclein and of dry egg-albumen was repeatedly administered to one bird, which had lost 25 per cent. of its weight and was very lame and unable to move after fifty days' feeding on rice meal with egg-albumen; the next day the bird was able to walk with considerable agility, to use its wings in normal fashion, and to feed itself, and had lost the continuous convulsive movements of head and limbs that had previously troubled it. Twenty-four hours' further treatment, and the bird was apparently fully recovered and able to walk and even fly. Six days later it was killed and no degenerated nerve fibres could be found.

Similar results were obtained in the case of a second bird, which was not killed, however, and so no examination of the nerves was possible. The third bird apparently completely recovered on a similar treatment, and was able to fly on the third day of treatment. It was killed, and the usual appearances were found after death—oedema of the muscles of the limbs, other muscles with diffuse haemorrhages, numerous nerve fibres with typical appearances of degeneration in the sciatic nerve. In the upper limb nerves no degenerated fibres were found, however, and the general condition of nutrition appeared good.

Dried pressed yeast is even more powerful a curative agent than yeast nuclein. Unless the bird dies before the yeast has passed from the crop, its administration results invariably in recovery if a sufficient amount be given.

Schaumann has tried this in a large number of birds, small amounts (0·1 to 0·3 gram) have no effect, but one gram a day is quite sufficient. In birds not severely lamed, and able to feed themselves, recovery is fairly rapid. On addition of yeast to the diet, the symptoms of lameness disappear in a day or two, the weight regains rapidly its level on mixed diet, and the degenerated nerve fibres progressively diminish in number and in the amount of degeneration. (Only a few typically degenerated fibres were found in a bird killed on the sixth day, and after fourteen days no degenerated fibres were found, but a few still show 'foam-structure,' the earliest stage of degeneration, the last of regeneration.)

Schaumann takes for illustration in his text one of these birds, which had lost 40 per cent. (120 grams) of its weight on an exclusive diet of polished rice and was so severely lamed as to be on the point of death; 3 grams of dried rice pap with 1 gram of yeast was forcibly administered (the dried yeast contains 4·25 per cent. P_2O_5). The bird could walk fairly well in twenty-four hours, and after a further administration of 1 gram of yeast with its rice, was again able to fly. Schaumann gives photographs of this bird taken on these three days, and the improvement is most marked, especially as this bird had (also the first one mentioned as cured with yeast nuclein, photographs of which are also reproduced) convulsive movements of the head and limbs, accompanied by spasm of limbs and neck muscles (leading to retraction of the head), a condition which is of even more immediately fatal import than a severe degree of lameness.

After these two days the bird was able to feed itself, and received rice pap with addition of 5 per cent. of dried yeast. Its improvement was marked ('visible'), and in twelve days added 44 per cent. to its weight (80 grams).

Katjang-idjo beans (P_2O_5 1·08 per cent.) exert an excellent curative effect; lame birds are sufficiently restored in forty-eight hours to run and fly, and their weight rapidly increases—(30 per cent. in eleven days, 17 per cent. in four days). The amount of the bean required is about 1·3 gram per day for each bird.

Dry yellow peas were almost as effective as Katjang beans, curing five birds very rapidly.

Many organic phosphorus-containing extractives of plants and

tissues were also used for curative purposes. Nucleic acids from yeast have already been referred to and will be remembered to have some power.

Yeast lecithin appeared to have no curative influence, but seemed to have some protective effect, for one pigeon was still alive after eight weeks' feeding with rice pap to which 0·5 gram of yeast lecithin per day was added, and though it had lost some weight no lameness appeared.

Ovolecithin, tried in one case, improved the condition of the lamed bird at first, but the good effect was not permanent, and Katjang-idjo lecithin similarly seemed to have an initial but only transient curative effect.

Commercial phytin and protylin were tried and found to be valueless.

Schaumann separated several extracts from Katjang beans—the pepsin-hydrochloric acid extract contained a large proportion of the phosphorus and was a powerful curative agent. Administration of one gram per day for two days, followed by 0·5 gram for seven days, completely restored one markedly paretic pigeon; and a similar result was obtained with a body of the phytin group extracted from this bean, two lame birds being obviously better the day after its administration, but continuation of the treatment did not avail to completely restore the birds, or indeed to keep them alive.

Testicular extract has a distinct protective power; two birds to which 1·5 grams per day each was given with the rice pap lived respectively forty-eight and seventy-five days, on the average twice as long as on rice alone. Both showed great loss of weight (54 per cent.) and neuritis, though one was lame for a short time only, and the other lived for thirty days after symptoms of lameness had appeared. The same extract administered to a paretic bird improved its condition and materially delayed its death (probably by about thirteen days).

Schaumann here calls attention to the complete 'protective' and 'curative' influence of this extract in the case of dogs.

Some signs and symptoms are present in all the birds which are receiving polished rice alone or with non-protective additions. Diarrhoea with thin excretae is one of the most noticeable, the colour of the stools being decidedly green—the stools contain only about

25 per cent. of the phosphate present on a full mixed diet. Loss of body weight is also constant, varying in amount from 25 to 54 per cent. In nearly all cases the birds showed loss of appetite in the later stages, remained sitting and were unable to fly.

The differences in duration of life were very considerable, not only with different nutriments (non-protective), but even in the same series—the greatest variation in the same series was from twelve to sixty-one days.

Equally marked were the differences in the degree of incapacity which developed before death. In rare cases scarcely any weakness of the legs was observable even just before death occurred, and the birds might even fly shortly before the sudden onset of death. In other cases all grades of lameness of legs and wings may appear, some are quite unable to fly, and can only move with difficulty, walking uncertainly, stumbling and falling forward.

Irregularity and galloping of the heart beat occurs in a fair number of lame birds.

The same variability was shown in the degree of degeneration of the nerves of different birds of the same series when examined after death.

Schaumann states that the grade of lameness is no measure of the amount and grade of degeneration in the nerves; marked lameness with slight degeneration, or slight lameness with marked degeneration being both observed.

In one case no difference could be made out in the degree of degeneration of the two sciatic nerves of a pigeon, one excised when markedly lame, the other obtained by killing the bird when apparently fully restored by two days' feeding on Katjang beans. In other birds, which were killed after being apparently completely restored from their lameness by one or two feeds of curative material, the nerves were obviously very degenerated in spite of the fact that the paresis had completely disappeared.

Schaumann suggests that the phosphatic bodies may in some way serve as sources of energy (physical or chemical) in the nervous system, and refreshment of the supply enables the central nervous system to overcome the hindrance of the degenerated nerves.

The researches on pigeons have been given in some detail, and we propose to only briefly note those carried out on other animals.

TABLE I

Experiment	No. of birds	Nutriment	Additions		Neuritis	Average loss of weight per cent.	Average day of death
I	9	Pap from polished rice (20 gm. per bird per day)	—		—	41	35
II	3	Pap from extracted polished rice	—		—	26	12
III	3	Raw polished rice	—		—	44	33
IV	8	Polished rice pap	Dried egg-albumen		—	37	28
V	5	,,	Mixed inorganic salts without P_2O_5		—	39	32
VI	4	,,	Mixed organic salts Calcium biphosphate	1 per cent. 1·6 per cent.	Marked lameness appearing 4 to 11 days before death	35	27
VII	10*	,,	Albumen metaphosphate	1·6 to 33 per cent. (contains 7·8 per cent. P_2O_5)	Marked lameness	29	23
VIII	4	,,	Calcium glycero-phosphate	1·6 per cent.	,,	33	19
IX	4	,,	'Scientific' yeast nucleic acid	1 per cent. (P_2O_5 5·54 per cent.)	More or less severe lameness	36	27
X	5*	,,	'Medicinal' yeast nucleic acid	1 per cent. (P_2O_5 16·3 per cent.) and 1·6 per cent. egg-albumen)	All much lamed in 15 days	33*	15*
XI	2	,,	Sodium nucleate (yeast)	1·5 gm. per bird per day (8 per cent. P_2O_5)	Lameness not marked	50	39
XII	2	,,	Testicular extract, (Bull)	1·5 gm. per bird per day (3·5 per cent. P_2O_5)	Lameness came on slowly, and was not extremely marked till just before death	54	61

* Several of these birds were used for experimental cure. Average figures are taken from remainder.

Guinea-pigs kept on polished rice or maize, with or without the addition of yeast, die during the fourth week; the loss of weight is between 20 and 40 per cent., and though lameness is not noticeable there are signs of early degeneration in the nerves. In addition, haemorrhagic lymphadenitis may develop in animals on yeast. In many animals, also, rarefaction of bones (osteoporosis) was very evident, and the bones might be as thin as paper.

Rats were kept on a diet of egg-albumen, starch (potato), sugar and inorganic salts, with or without phosphate (albumen-metaphosphate); death occurred in about 40 days without apparent lameness; on fine rye bread, denaturised by heating with soda, they die in 63 days (average), losing 47 per cent. of their weight.

Rabbits.—Maize, and maize with egg-albumen, is inadequate: death occurs in 45 days, with slight degeneration, and the loss of weight is about 30 per cent; other symptoms are lameness and loss of reflexes in the hind limbs, loss of hair, loss of appetite and activity; they die of exhaustion or convulsions. One strong rabbit lived after ninety-seven days' feeding on rice and egg-albumen.

Maize with the addition of 4 per cent. of dried yeast is fully adequate and maintains the animals in normal health.

Katjang-idjo and peas rapidly restore animals which have become paretic—the lameness vanishing and the body weight rising rapidly.

An Ape, which thrives on rice pap, currant bread, nuts and fruit, died with paresis and loss of nearly 30 per cent. of its weight after seventy-four days' feeding on pap from polished rice previously extracted with water. There was little typical degeneration, but nearly all the nerve fibres showed 'foam-structure.'

In the next set of animals, *denaturised horse flesh* was used as the basis of diet. The flesh is heated in an autoclave to 120° C. with dilute soda-solution—the alkali being afterwards neutralised with hydrochloric acid. The nuclein molecule is probably split in the process. The proteids are not apparently affected. (Neither ammonia nor sulphur bases are formed.)

Rats, on denaturised flesh, become paretic in about a month, but the illness does not progress, and the experiments were abandoned after three months or more. Loss of weight, about 5 per cent.

Cats.—Denaturised flesh: death in about 50 days; loss of weight, 30 per cent. Lameness present before death—slight nerve changes demonstrated.

Dogs.—Denaturised flesh (about 1 kg. a day); loss of weight, 25 per cent; death, 50 days (average). Severe lameness starting in the hind limbs is the first symptom, and progresses to complete incapacity; death occurs with convulsions. In one case there was *sponginess and haemorrhages* in the mouth and gums, with ulceration; nerve degeneration is only slight. One dog lived for fifty-five days and was kept quite fit and well by the addition to the diet of four grams a day of testicular extract; in the earlier stages with larger amounts (six grams) of extract he put on additional weight to the extent of 11 per cent. (1,200 grams), showing that the horse flesh is not deficient in caloric value, but the testicular extract (P_2O_5 3·36 per cent.) supplies some essential substance. The testicular extract was replaced on the 56th day by 5 grams of yeast, with the result that the dog gained 1,150 grams (15 per cent.) in weight in thirty-one days and was very well and active.

Though dogs die in 50 days on the average on denaturised flesh, this one lived three months in full health, when some five grams a day of these substances (rich in phosphorus) were added. He was now kept on denaturised flesh alone, and in 50 days had lost 2,550 grams and was severely paralysed. Four days later, when death was apparently imminent (the pulse was 270), ten grams of fat-free yeast were administered—the dog improved and was able to move in twenty-four hours (pulse now 100). For three further days five grams of yeast were given and the dog had completely recovered and was visibly fatter.

Similar rapid cure was obtained in other cases with testicular extract—one animal gained a kilogram in six days after treatment was begun.

Schaumann emphasises the fact that the symptoms and changes in these animals are similar to those found in sailing ship Beri-beri, and that in patients with that disease, as in the animals, the reaction to a more suitable diet is exceedingly prompt.

Tropical Beri-beri differs more particularly in the length of time required for recovery, which is often many months.

By feeding a goat on rice and maize till lameness and loss of weight were marked, and repeatedly reviving it with Katjang beans and yeast, either in single doses or continued for short periods, Schaumann prolonged an experiment for six months. The goat

was at the end very weak and paralysed, and was slow in reacting to curative treatment. The animal was killed and extremely careful examinations made of its organs. Degenerative changes were present in the nerves and some wasting was also shown in the columns—no alteration of the cellular elements was demonstrable. Some alterations were detected in the vagi, and the muscles were oedematous and markedly rich in nuclei.

The appearances and symptoms were suggestive of those of Tropical Beri-beri.

Schaumann gives full details of his various experiments on animals, with an account of the results of the post mortem and microscopic findings and tables of averages. He also gives the results of exhausitve analyses of all the substances used for feeding in these experiments, with special references to the amounts and characteristics of the different classes of phosphorus compounds present in them, and to various extracts obtained by solvents (acids, alkalies, etc., peptic and pancreatic digestion).

He draws the following conclusions from his experiments on animals.

CONCLUSIONS

I. Food stuffs which lead to the development of Polyneuritis in animals are characterised by a low content of phosphorus or of certain organic compounds of phosphorus. This may be either fundamental or be caused by artificial processes.

II. Animals are not protected from the ill effects caused by such diets by the addition thereto of proteids, inorganic salts, inorganic phosphates, or the synthetic organic compounds of phosphorus (calcium glycero-phosphate, albumen-metaphosphate).

III. The addition of certain substances, rich in organic phosphorus, to such diets exercises both a protective and a curative effect. Yeast, rice meal, wheat bran, peas, Katjang beans, and testicular extracts are the chief substances with this power. Carnivora and herbivora, however, react rather differently to testicular extract, the former are completely protected, the latter only in a less degree.

IV. Artificially separated organic phosphorus compounds of various kinds, prepared from these natural protective substances, exercise only a moderate and transient influence. Such compounds

include yeast nucleic acid, phytin-like compounds from Katjang, phytin from rice meal, and possibly certain phosphatides.

V. Apparently the protective or curative effect of these substances is dependent not on any one of their organic compounds of phosphorus, but on the collective effect of a number of these. Animals do not apparently possess the power of forming the organic phosphorus compounds necessary to their economy from inorganic phosphates by their own metabolism, but are dependent for their provision on the plant world, as they are for other classes of foodstuff (e.g., protein and carbohydrate).

VI. The metabolism of phosphorus and nitrogen stand in close relationship to one another.

VII. Spontaneous or experimental polyneuritis in animals appears to be a disease of metabolism, attributable to the lack of some specific organic phosphorus compounds whose identity is still uncertain.

SAILING SHIP BERI-BERI

Turning now to the subject of Ship Beri-beri, Schaumann points out that this disease generally occurs on sailing ships on the return voyage, that it does not usually attack the majority of the crews, and that change of diet, particularly fresh meat and vegetables, exercises a rapid and complete curative effect. (Crews are mainly Europeans.)

In his tables are given the logs of the voyages of ten sailing ships, the incidence of Beri-beri among their crews, the provender and its appearance, and the men's statements as to the cooking and palatableness of the various articles of diet. In a second set are analyses of the various foodstuffs from the ships and others of fresh foodstuffs for comparison; these show the alterations in proteid, etc., due to preservation and long storage, more especially with reference to their phosphorus contents; further examinations were made of the substances after cooking, again with special reference to the behaviour of their phosphorus and eatability; the moulds present were also examined. In other tables are given the results of analyses of the excretae of Beri-beri patients and the results of experiments on animals with the provender from the ships, etc.

He notes first that the phosphorus excretion of the patients is

exceedingly low (50 per cent. of normal), and that it rapidly rises on phosphorus rich diets but some retention of phosphorus occurs.

He points out that the various customary articles of diet carried on sailing ships fall into two groups: rice, white bread, and potatoes, etc., in the one group, poor in phosphorus, are known to give rise to neuritis in animals.

Of the other group of substances, rich in phosphorus, the salt meat has been shown to lose nearly 50 per cent. of its phosphorus by the combined action of the pickle lye and of the water used in boiling, König (1904). Schaumann's results confirm this loss in the meat from the Beri-beri ships, and further show the presence in the lye of purin bases, which must arise from breakdown of nucleo-proteids.

The peas and other legumes are found to be usually mouldy, are hard and resistant to cooking even with soda. They are frequently quite uneatable, and many Beri-beri patients turn against them, and Schaumann further shows that their phosphatides seem to have undergone considerable changes.

Preserved vegetables carried on one ship were very rich in organic phosphorus, and had been found to be extremely valuable in the treatment of the sailors stricken with Beri-beri. Preserved meat is also rich, particularly in lecithin phosphorus.*

Schaumann considers that the cause of Beri-beri on ships lies in the lack of organic phosphorus compounds in the diet of the sailors. This is especially noticeable on the return voyage (especially in ships loading home from nitrate and guano ports where fresh meat and vegetables are not obtainable), in part owing to the sailors being partly driven to bread, rice, etc, through the mouldiness of the peas or their hardness from keeping (rendering them impossible to soften even when boiled in soda), or the decay and smell of the meat. To this is added in other cases the loss of organic phosphorus in the salt meat due to the action of the lye, and the similar loss in the leguminous foods owing to standing or to boiling with soda.

He calls attention again to the affinity between scurvy and ship Beri-beri (*vide* also his animal experiments), and suggests that scurvy may be found to be due to use of stale vegetables, and Beri-beri to use of rotten or stale flesh—the important factor in each being lack of organic compounds of phosphorus.

* Schaumann does not appear to note that this article must often be denaturised by heating to 120° in preserving. (Holst, *loc. cit.*)

As a curative agent, he says all sailors know the value of fresh meat and vegetables. As a preventive agent, he suggests that it might be possible to carry dried yeast, rice meal or testicular extract for use when the ordinary diet becomes inadequate, and especially lays stress on the probable value of requiring all ships engaged on voyages where fresh provender may be difficult to obtain, to carry a supply of Katjang beans in sealed (sterilised) cases. In the future, he hopes a more active principle requiring but small bulk may be isolated.

Sailing ship Beri-beri is a disease of metabolism dependent on lack of organic phosphorus in the diet.

TROPICAL BERI-BERI

Schaumann's observations on this disease are in part based on the conclusions of workers in the East, and in part on clinical and therapeutic observations on patients in the Hospital at Hamburg. Tropical Beri-beri contrasts with sailing ship Beri-beri in being chiefly observed among the Chinese stokers, trimmers, etc., of steamships, who cook for themselves and keep to their accustomed dietary even when on board. The dietary is usually frugal, considering the hard work they perform.

Fletcher and others have shown that Tropical Beri-beri is connected with the long continued use of a predominating amount of polished rice in the diet (uncured rice is used synonymously with polished rice). Grijns and others have shown the same connection of polished rice with polyneuritis, and rice meal or its alcohol extract (Fraser and Stanton) prevents the occurrence. Breaudat (1910) has demonstrated that rice meal (the part removed in polishing) has a similar curative and prophylactic influence in Tropical Beri-beri.

Analyses of the total mixed diets of Fraser, Fletcher and Ellis show that they are quite ample in carbohydrate, proteid and fat, and in caloric value for the requirements of men, judging by ordinary physiological standards, and the development of Beri-beri cannot depend on lack of these substances. On the contrary, the diets containing polished rice and causing Beri-beri contain only about two-thirds of the phosphorus requirements (4·5 grams) of a man, even

if a high average value is taken as a basis of calculation, and must often be less than this figure. The process of cooking may further reduce this. The pericarp which forms the difference between Beri-beri rice and wholesome rice is specially rich in organic phosphorus and in fat, but contains no other peculiar substances as far as can be determined. Grijns showed that the fat is not the important principle, and so we are left only with the phosphorus compounds.

Schaumann gives analyses showing that the urinary excretion of phosphorus in patients with Beri-beri is much below the normal (average 50 per cent.), and this is accompanied by an almost equal diminution of urinary sulphur and nitrogen. Previous observers found even larger differences (70 per cent.). Addition to the diet of substances rich in phosphorus leads to a marked rise in the excretion of all these bodies.

Aron (1910), by careful experiments on the metabolism of a Beri-beri patient extending over four weeks, showed that he was unable to obtain sufficient phosphorus and nitrogen on the diet used before the attack to meet his requirements, which were higher than is normal. Remedy of the defects resulted in rapid cure. Aron further showed that outbreaks of Beri-beri on ships often occurred about six or eight weeks after the substitution for that previously used of a rice with a much lower phosphorus content—possibly only one-third of the previous, the rest of the diet remaining unaltered.

Hirota (1900) has shown that nursing children (in Japan) often develop severe Beri-beri two or three weeks before their mothers, and improve rapidly on change of milk. This is associated with a great diminution of the phosphorus (especially in organic combination) of the milk, and would seem to be associated with phosphorus hunger in the mother, resulting in a retention and sparing of phosphorus.

Hulshoff Pol (1910) has demonstrated that Katjang beans form, with regard to polyneuritis in animals, a *certain and rapid* cure for Beri-beri, and extract of the beans is equally efficacious. He further showed that addition of a similar amount (150 grams) to the dietary of various pavilions in an asylum absolutely abolished Beri-beri; vegetables were less effective. The staple diet in the asylum consisted of polished rice.

This has been confirmed by many others, and though the chronic lesions of Beri-beri usually disappear very slowly under treatment,

occasionally the paralysis disappears with the startling rapidity observed in the experiments on animals.

Schaumann and Werner tried the therapeutical effects of phosphorus-rich compounds on Tropical Beri-beri and obtained improvement by the use of yeast, nucleic acid, testicular extract, etc., though the improvement was not so marked as in polyneuritis in animals, owing to the more advanced lesions in these cases requiring longer for regeneration. (Fat-free yeast and carefully prepared nuclein should alone be used.) Katjang beans, peas, and also the peptic extract of Katjang are also very valuable.

On these grounds it appears clear that Tropical Beri-beri resembles experimental Polyneuritis and Ship Beri-beri in being due to lack of organic phosphorus in the diet, but *it appears to be due to a chronic deficiency of long duration, with severe deep-seated lesions requiring a long time to cure.* The experimental neuritis in a goat already described is analagous to Tropical Beri-beri. The other experimental cases and Ship Beri-beri, on the contrary, are due to a sudden large deficiency of organic phosphorus, and the lesions, though severe, are not deep-seated, and are rapidly recovered from.

In the majority of cases, *Beri-beri is due to a gross deficiency of the organic phosphorus in the diet.* In other cases the differences may be individual in the patient, since some individuals require much larger amounts than others, and others again may be unable to absorb and assimilate the compounds, though present in the diet. Schaumann quotes the case of a German colonist, who, after being very ill with malaria on the Amazon, developed Beri-beri on the voyage home though on an ample diet; in this case the intestinal absorption appeared faulty, as there was a great deficiency in urinary, and a very large increase in the faecal phosphorus.

Occasionally, epidemics of Beri-beri appear to be due to a bacterial infection of the gastro-intestinal tract, either through the catarrh interfering with absorption or to the bacteria (or their products) absorbing or splitting the organic phosphates before they could be absorbed.

Tropical Beri-beri is a disease of metabolism due to the amount of organic phosphorus compounds assimilated being below that essential for the human organism.

This is in the majority of cases due to deficiency of organic phos-

phates in the diet, but may in occasional cases be due:—(*a*) to deficient absorption of organic phosphates by the alimentary tract, or (*b*) to bacterial infection possibly splitting the phosphates before absorption or interfering with the absorptive power of the intestine.

CONCLUDING CONSIDERATIONS

A whole cycle of other diseases in all probability have a similar etiology to that of Beri-beri, more especially Ship Beri-beri. Scurvy has already been conclusively shown to be of this nature by a whole series of observations, and to all appearance Infantile Scurvy, Rickets, and Osteomalachia are also included. Pellagra and the form of malnutrition described by Czerny and Kellner in artificially fed children may be further examples.

The suspicion arises that all these diseases may be due to deficient assimilation of organic phosphorus, but it is difficult to understand how a similar cause can produce the different effects shown by the symptoms in these diseases.

It has already been shown that the animal organism is not adapted to build up organic phosphorus compounds for itself, but depends for these on the plant kingdom, and it is probably equally unable to form compounds of one group from those of another (e.g., nucleo-proteids from phosphatides).

Since different groups play different rôles in the economy of man, it is certain that deficiency of each group will have its own special effect, and in addition this will be complicated by an associated influence on the general metabolism; for example, inorganic compounds would react on the alkaline earths of the bones, nucleo-proteids would react on the proteins, etc.

Individual differences would further intervene to complicate the picture, more especially age differences, and infancy, childhood, puberty, pregnancy and old age would, in particular, have a far-reaching influence.

So by deficiency of single groups, or of combinations of groups of phosphorus compounds, various symptom complexes—different diseases—may be originated. Thus lack of nucleo-proteid in adults is probably connected with Beri-beri; in children a similar deficiency might give a different disease: deficiency of phosphatides may give yet a third, a combination of the two will give yet another picture.

Application of the principles already applied to Beri-beri in this paper may bear fruit also in these diseases, though only after long and complicated researches may the answers be obtained.

Schaumann hopes that his speculations may lead to encouraging further researches along systematic lines. While quite recognising that they are but hypotheses resting on slight grounds, yet the advance of science and medicine would be but slow were it not largely aided by experimenters, who have striven with all the means in their power to prove or disprove theories based on even slighter grounds; and Schaumann has felt it right not to conceal his speculations, as they may yet bear fruit.

We feel that this monograph is so thorough and complete, and so well thought out, as to deserve communication at considerable length, especially as recent occurrences have shown us that it, like the previous researches of other workers on similar lines, has not become as widely known in physiological, scientific and medical circles in this country as it would had it not been published in a journal mainly concerned with Tropical medicine. We think that all readers will agree with us that Dr. Schaumann is to be congratulated, not only on his important contribution to our knowledge, but on its arrangement and its literary merit.

To our nation, with its wide shipping interests and tropical possessions, it is the more important, and we must congratulate ourselves that it is largely based on the work of British medical men and scientists in our Eastern possessions. Their work has already had widespread influence in checking the heavy incidence and mortality of Beri-beri.

In 1910, Fraser and Stanton published a further paper carrying on their work on these lines, and have further confirmed the association of organic compounds of phosphorus with the deficiency of diet causing Beri-beri.

Our own researches as far as they have extended are in full agreement with those of Schaumann, though results have as yet only been obtained with our experiments on pigeons.

We can confirm the unfavourable influence of polished rice, steamed rice, and steamed barley fully; and the protective influence of whole rice, whole barley, rice meal, yeast and Katjang-idjo. The curative effects of yeast in pigeons severely affected with

neuritis were even more marked than we had expected, and we were astonished at the rapidity and completeness with which the birds recovered.

It was not our intention to communicate any of our results till considerably more experiments and analyses had been concluded, nor indeed to abstract previous work at such length as we have done.

The great public attention, however, which has been directed for some months to the question of our bread, led us to call attention to this literature in a letter to the British Medical Journal of May 6th, and now to communicate in detail those of our results which bear on this problem. We had so many enquiries for references that we thought it right to quote the previous work at some length, since the problem of the influence of rice in Beri-beri is so closely allied to that of a standard bread.

It is interesting to remember that the Germans refer to Rickets as the English disease, and to reflect that it is far more common in this country than it is in Germany, and further that the Highlanders and our Irish peasants are in large measure free from it.

Yet the children of all these races are brought up largely on similar diets (excepting such as are bottle-fed from the start). In the poorer classes of all, milk forms some part of the diet; in the peasant class it is usually good, in the town children it is often, however, neither abundant nor containing cream. Besides this, the children get their main nutriment from the national bread and from rice. Polished rice is used in all the nations, but the Highland child gets porridge from oats, the Irish child potatoes, the German rye bread, and the English child white wheat bread.

The organic phosphates are undoubtedly present in the oatmeal (0·9 per cent. P_2O_5) of the Highlander, and in the potatoes (unless deeply peeled); in the rye bread the organic phosphorus appears to be diffused through the whole grain (P_2O_5 1 per cent.), and even fine rye bread does not originate polyneuritis in fowls (Holst) or in rats (Schaumann).

In the fine English white wheat bread, however, the phosphorus has been removed with the bran, and is used largely (as is rice meal) as one of the best possible foods for fattening cattle. White wheat bread (P_2O_5 0·2), as Holst has shown, causes polyneuritis in fowls and Schaumann shows the existence of the important protective phosphates in the wheat bran.

Our own researches fully confirm Holst's results; indeed, the effects were more markedly deleterious than he found. The bread was a white flour bread, guaranteed to be made from the finest white flour, unbleached and unadulterated. Well nourished pigeons when limited to this bread devoured it greedily, but failed to flourish. Diarrhoea, and loss of weight, early commenced; listlessness and lameness followed shortly after. The first bird died on the 15th day, and others on the 16th and 20th days, showing marked degenerative changes in their peripheral nerves. Several were revived when extremely weak and nearly ready to die, showing severe lameness and, in some cases, the convulsions and retraction of the head described by Schaumann; but the average duration of life (allowing one or two days for the revived birds) was 29 days, and the average loss of weight 26 per cent.

Far different is the picture on Standard or whole-meal bread. The birds continue active and well, maintain their weight, clean and plume themselves; they remain able to fly and walk, and at the end of seven weeks were all perfectly well and had, on the average, gained 8 per cent. of their original weight. On whole-meal bread in two cases, pairing occurred; one pair successfully hatching the two eggs (though the diet was changed to a mixed diet during the sitting); the other pair were put on white bread, broke the eggs and began to go downhill.

These results are given more completely in the tables at the end of this article, and appear to us, in conjunction with Holst's, Leonard Hill's (B.M.J., April 30), and Schaumann's, to fully confirm the claims of the advocates of Standard or whole-meal bread.

We have seen that some degree of rickets is almost universal among the children of our poorer classes, who, in addition to lack of sunlight and fresh air, live largely on white wheat bread, and that it is not so prevalent in those nations whose children eat porridge (oatmeal 1 per cent. P_2O_5), or rye bread, with a higher content of organic phosphorus compounds. We know that they often get little else but poor milk and *margarine* (P_2O_5 0·03 per cent.), which may also be deficient in similar compounds, and we see that marasmus, diarrhoea, oedema of the limbs, spasm and convulsions (especially tetany) are common to rickets and to the experimental neuritis of pigeons and animals.

It seems to us that Schaumann's hypotheses are not likely to long

lack justification with regard to this disease and also to its close ally, infantile scurvy, though the symptom complex is probably complicated by secondary effects on digestion and on the metabolism of lime and proteid.

It may be objected that our white bread is baked with yeast and so the missing organic phosphates are compensated for, but the amount of yeast used is very small and probably insufficient (Holst's pigeons died on yeast bread towards the end of three months; more slowly, it is true, than on bread baked with baking powder (average 40 days), but none the less surely). And it must further be remembered, that much of the bread now sold is made with baking powder and not with yeast, and so a further factor making for deficiency is introduced. Failures of absorption, bacterial infections, and other internal disorders no doubt play their part as in Tropical Beri-beri, but it may well be that success of the present agitation for a wholemeal bread will have a wide reaching effect on the betterment of the physique of our nation, in lowering our death rate and in lightening the overcrowding of our hospitals.

The following tables include the results of experiments referred to in the text. Some of the series are merely confirmatory of the results of previous observers; others refer to the experiments with white and Standard bread. The results of curative treatment with yeast and other substances are also briefly alluded to, and in a final table the results of analyses (E. S. E.) of various substances used are given.

We hope in a future contribution to give the results of our attempt to isolate the active principle, whether it be one of the organic phosphorus compounds or a substance which associates itself with these in its reactions, as do ferments with nucleo-proteids. We wish at the present time to express our indebtedness to Professor Sherrington, Professor Moore, Miss Tozer, and others for their kindness in advising and assisting us in various chemical and neurological problems that have confronted us.

In conclusion, we wish to emphasise the great importance of these investigations to a country such as this with wide Shipping and Colonial interests, and hope that an appreciation of these facts will lead to the adoption of the necessary additions, where such diets are largely used, as has already been done in the Straits Settlements with striking results.

TABLE III

Series	No. of Pigeons used	Nourishment	Average weights in grams					Percentage difference of weight		Average day of		Remarks
			1st day	8th day	15th day	22nd day	End	—	+	Marked incapacity	Death	
A	6	Uncleaned rice (Paddy)	342	364	375	369	373	...	9	...	...	Expt. abandoned 56th day, all quite healthy
B	6	Polished rice (known to have caused Beri-beri)	329	284	236	206	201	39	...	32	34	
D	6	Polished rice (from a Liverpool shop) ...	316	268	226	[illegible]	180	43	...	32	35	
l	3	Uncleaned rice (denaturised at 120° C)	350	312	276	251	251	28	...	19	22*	*See protocol
m	3	Denaturised rice + 2 grams dried yeast per bird daily	369	377	335	374	374	...	1	...	...	Expt. abandoned 35th day, all quite healthy
n	3	Denaturised rice + 2 grams denaturised yeast daily	324	295	259	224	206	36	...	22	25*	
E	6	Barley grain	334	344	352	367	364	...	9	...	...	Expt. abandoned 30th day, all quite healthy
F	6	Barley grain (denaturised at 120° C.) ...	367	378	343	289	198	46	...	33	37	
C	6	Polished rice + mineral phosphates ...	350	346	298	275	256	27	...	36	38	
a	5	Standard bread (yeast 0·3 per cent.) ...	372	375	381	378	384	...	3	...	...	Expt. abandoned 50th day, all healthy
d	6	Wholemeal bread (yeast 0·3 per cent.)...	328	306	365	342	339	...	3	...	...	Expt. abandoned 36th day, all healthy
b	5	White bread (yeast 0·3 per cent.) ...	348	324	283	258	245	30	...	27	32	
c	6	White bread (yeast 0·3 per cent.) ...	347	349	306	276	...	21	...	18	20	

TABLE III.—Successful Restoration Experiments

Series	No.	Previous diet	Percentage loss of weight	Day of treatment	Type of neuritis	Treatment	Results
C	2	Polished rice	31	40 *et seq.*	Peripheral	Yeast	Improved 24 hours. Flying 48 hours.
C	4	Polished rice	29	34 ,,	Vestibular	Yeast	Improved 24 hours. Flying 48 hours.
D	1	Polished rice	39	29 ,,	Vestibular	Maize and Soya oil	Improved 3 days. Weight regained, 14 per cent. in 3 weeks
D	3	Polished rice	25	29 ,,	Vestibular	Maize and Soya oil	Improved 3 days. Weight regained, 20 per cent. in 3 weeks
F	2	Steamed barley	39	28-31 day	Vestibular	Yeast (3 days)	*Full recovery 3 days.* Relapsed later, on omission of yeast
I	1	Steamed rice	24	16 *et seq.*	Vestibular	Yeast	Improved rapidly. Flying 3 days
I	2	Steamed rice	36	19 ,,	Vestibular	Yeast	Improved 24 hours. Flying 48 hours
I	3	Steamed rice	24	28 ,,	Slight peripheral	Yeast	Improved 2 days. Weight regained, 13 per cent. in 2 weeks
n	2	Steamed rice : steamed yeast	24	27 ,,	Peripheral	Yeast	Improved rapidly. Flying 48 hours
n	3	Steamed rice : steamed yeast	32	25 ,,	Vestibular	Yeast	Improved rapidly. Flying in 3 days
b	2	White bread	36	43 ,,	Peripheral	Katjang	Improved 24 hours. Weight regained, 19 per cent. in 7 days
c	1	White bread	25	21 ,,	Peripheral	Yeast	Regained 17 per cent. weight in 7 days
c	3	White bread	18	20 ,,	Peripheral	Yeast	Regained weight competely in 2 weeks
c	4	White bread	16	23 ,,	Vestibular	Yeast	Flying in 3 days. Weight regained, 15 per cent. in 7 days
P	2	Rice and lecithin	29	21 ,,	Peripheral + Vestibular	Standard bread	Improved slowly. Lameness continued for 7 days
Q	1	Rice and nuclein	32	20 ,,	Vestibular	Standard Bread	Improved rapidly. Feeding in 24 hours. Weight regained, 19 per cent. in 7 days
Q	3	Rice and nuclein	33	23 ,,	Vestibular	Standard bread	Improved rapidly. Feeding in 24 hours. Weight regained, 18 per cent. in 7 days
S	1	White bread	28	49 ,,	Peripheral + Vestibular	Yeast	Flying, walking, and feeding in 24 hours

degeneration could be discovered in small cutaneous nerves, in the anterior and posterior spinal roots, in the vagi, or in the vestibular nerves in spite of careful search in several cases. Nor did any degeneration appear to be present in the spinal cords.

Series l and F

The rice and barley grain used in experiments A and E was also used in these series, but was previously exposed to a temperature of 120° C. for two hours in an autoclave.

The birds on steamed rice failed very rapidly, marked convulsive neuritis occurring on the 16th and 19th days respectively, and the birds would certainly have died in the course of forty-eight hours. Forced feeding with ordinary brewers' yeast (and rice) was instituted, and in both instances the birds were free from convulsions and able to walk in twenty-four hours, and in a further day appeared quite normal.

Professor Sherrington kindly examined one of these birds for us before and after the first twenty-four hours of yeast feeding. Before treatment started it could barely stand and held the head completely retracted; on attempting to walk it became convulsed and turned a series of back somersaults till brought up by some obstruction; the wing reflex (flap reflex) was absent, and the eye and head reflexes were abnormal. The next day it could walk and fly readily, the wing reflex and head reflexes were normal, and all signs of labyrinthine trouble had disappeared. The third bird became very weak on the 24th day, and was barely able to move or feed itself. It rapidly recovered on yeast feeding and change of diet. In Table I, in reckoning the probable day of death, an ample margin has been allowed.

The birds on denaturised barley failed slightly less rapidly, the first died with convulsive seizures on the 22nd day, the last on the 45th day. One was restored on the 28th day when very near death by yeast feeding, which was continued for three days. It failed again on the barley diet and died on the 44th day.

Series m

Shows the absolute adequacy of a diet of denaturised rice, with the addition of one gram dried yeast per bird per day.

SERIES n

Shows that the protective influence of the yeast is entirely destroyed by heating to 120° C. The birds showed typical neuritis and other symptoms usually associated. Two rapidly recovered on treatment with ordinary yeast.

PERCENTAGE OF P_2O_5 IN VARIOUS DIETS USED :—

		Percentage of P_2O_5
Polished rice	6 samples	0·26
Rice meal	4 ,, ...	2·75
Uncleaned rice	3 ,, ...	0·61
Dried yeast	4 ,, ...	4·03
Katjang beans	2 ,, ...	0·95
Barley grain	3 ,, ...	0·92

REFERENCES

ALBU AND NEUBERG (1906). Phys. und Path. des Mineralstoffwechsels, Berlin.

ARON (1910), Berl. klin. Woch., No. 21, p. 995.

BRADDON (1909). Brit. Med. Journ., p. 1007.

BREAUDAT (1910). Bull. Soc. Path. Exot., Jan. 12, p. 13.

EIJKMAN (1897). Virchow's Arch., CXLVIII, p. 523.

FLETCHER (1907). Lancet, June 29, No. 4374, p. 1776.

FRASER AND STANTON (1909). Arch. f. Schiffs- und Trop.-hyg., XIII, Beiheft 6, p. 82.

FRASER (1909). Lancet, XXVI, 4459, p. 451.

FRASER AND STANTON (1910). Lancet, No. 4515.

GRIJNS, G. (1908). Geneesk. Tijdsch. v. Ned. Ind., XLVIII, 5. pp. 680-704.

HIROTA (1909). Zentralbl. f. inn. Med., p. 273.

HOLST, AXEL AND FRÖLICH (1907). Journ. of Hyg., Oct., VII, 5.

HULSHOFF POL, J. (1910). Arch. f. Schiffs- und Trop.-hyg., XIV, Beiheft 3.

KÖNIG (1904). Chemie der Menschlich. Nahrungs- und Genussmittel, Berlin.

NOCHT, B. (1908). Arch. f. Schiffs- and Trop.hyg., XII, Beiheft 5, pp. 115-130.

SCHAUMANN, H. (1908). Arch. f. Schiffs- und Trop.-hyg., XII, Beiheft 5, pp. 137-157.

SCHAUMANN, H. (1909). Arch. f. Schiffs- und Trop.-hyg., XIII, Beiheft 6, 82.

SCHAUMANN (1910). Arch. f. Schiffs- und Trop.-hyg., XIV, Beiheft. 8.

Volume V December, 1911 No. 3

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY

THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Editor

PROFESSOR SIR RONALD ROSS, MAJOR I.M.S. (RET.), D.P.H., F.R.C.S., D.Sc., LL.D., F.R.S., K.C.B

In Collaboration with

J. W. W. STEPHENS, M.D., CANTAB., D.P.H.
R. NEWSTEAD, M.Sc., A.L.S., F.E.S., Hon. F.R.H.S.
J. L. TODD, B.A., M.D., C.M. McGill, D.Sc., Liv.
H. WOLFERSTAN THOMAS, M.D., C.M. McGill.
ANTON BREINL, M.U.DR.

C. Tinling & Co., Ltd.
Printers to the University Press of Liverpool
53 Victoria Street

TABLES OF STATISTICAL ERROR*

BY

PROFESSOR SIR RONALD ROSS, K.C.B., F.R.S.,

AND

WALTER STOTT,

HONORARY STATISTICIAN, LIVERPOOL SCHOOL OF TROPICAL MEDICINE

(*Received for publication* 24 *October*, 1911)

CONTENTS

I. EXPLANATION

1. Proportionately Small Samples .....page 347
2. The Correct Procedure 353
3. Proportionately Large Samples ..... ,, 356
4. Things of One Class..... ,, 359

II. TABLES

I. EXPLANATION

1. PROPORTIONATELY SMALL SAMPLES

These Tables have been specially constructed for practical use in sanitary, pathological and clinical work.

Suppose that we are studying things of any nature, such for example as men, cases of sickness, insects, leucocytes, trypanosomes, seeds, stones, etc., and wish to ascertain what proportion of all of them belong to a particular class, that is, have a given characteristic. Then we can answer this question with absolute certainty only in one way, namely, by examining the whole number of such things that exist in the region under consideration. But this is generally impossible; and we must, therefore, content ourself with examining only as many of the things as we can—ascertaining what proportion of these possess the given characteristic, and thence *inferring* what probable proportion of the same things in the region under consideration belong to the same class.

* To be obtained as a separate publication for two shillings and sixpence, postage included, from the Clerk of the Laboratory, School of Tropical Medicine, University, Liverpool. *All rights reserved.*

For example, if we wish to know with certainty how many people of any nationality have blue eyes, then we must examine all the people of that nationality. But this will be impossible. Hence we must examine 10, or 100, or 1,000 or more of the people; ascertain how many of *these* have blue eyes; and then *infer* from this sample what proportion of the whole nation are *likely* to have them.

Everyone knows, of course, that (provided the methods of examination are always equally careful and trustworthy) our estimate is more likely to be near the truth if we examine a large sample than if we examine only a small one—that is, if we examine many of the things than if we examine only a few. For instance, it would be absurd to attempt to estimate the proportion of persons with blue eyes by examining only five or ten persons. We should come nearer by examining one hundred or one thousand, and so on; but we should reach absolute certainty only by examining all the people in the country. The important question now arises: How many of the things must we examine in order to become *reasonably sure* that our result is within a *given percentage* of the exact truth? The labours of mathematicians enable us to answer this question, and the following Tables enable us to answer it in most cases without calculation. We must begin by dealing with the case in which the total number of things is so large that we cannot take for a sample more than a very small proportion of that total number. Hence the heading of this section is Proportionately Small Samples.

First, however, we must understand what exactly we mean by the phrases *'reasonably sure'* and 'a given *percentage* of the truth.' Both these phrases contain ideas of *degree*. Thus, if we are carrying out a strict scientific enquiry, we may wish to be able to say that the betting (or probability, as it is called) is 99,999 to 1 that our result is within one per cent. of the truth. We must then consult Table A 1, under the second column. Or it may suffice to say that the betting is 99,999 to 1 that our result is within five per cent. of the truth; and we then consult the same table under the sixth column. But it may happen that we can afford to be content with a lower degree of probability than this—it may suffice to say that the betting is only 99 to 1 that our result is within 3 per cent. of the truth; and we then look at Table A 4, under the fourth column. Lastly, we may be allowed to content ourself with an 'even chance' or

'toss up'—that is, a probability of 1 to 1; and for this we consult Table A 6.

The reader will now be easily able to understand the tables. The figures in the heading of each give the degree of sureness (so to speak) with which we may rely upon the inferences drawn from the table. Thus the odds are 99,999 to 1 that the inferences to be drawn from the first table are sound; or, in other words, out of 100,000 trials of the table only one is likely to be wrong. Table A 5, however, is likely to be wrong once in ten trials; and Table A 6, once in two.

The percentages at the head of each column give the percentage of 'statistical error'—that is, the amount by which the truth is likely to diverge from our observed result. Thus, if our result is 70 per cent., and the statistical error is 5 per cent., then the odds (as denoted by the figures 99,999 to 1, etc.) are that the truth will lie anywhere between 70 + 5 per cent. and 70 − 5 per cent.—that is, between 75 per cent. and 65 per cent. If, however, the statistical error is only one per cent., then the truth is likely to lie between the narrower limits, 71 per cent. and 69 per cent. If the observed result, *plus* the error, exceeds 100, or if the observed result, minus the error, is less than 0, we conclude that the number of things hitherto examined is not yet large enough to yield a useful result.

The figures down the first (left hand) column of each table refer to the percentage of the observed result up to 50 per cent.; and the figures on the same line in the body of the table give the total number of things which must be examined in order to ensure that that result will lie within the percentage of error given at the head of the corresponding column, with the degree of probability given at the head of each table. Thus, if the observed result is 43 per cent., we must examine 47,780 things to ensure that the odds are 99,999 to 1 that the truth lies between 44 per cent. and 42 per cent. But we need examine only 1,328 things to ensure that the betting is 99,999 to 1 that the truth lies between 49 per cent. and 37 per cent. We must examine 26,541 things to ensure, with a probability of 999 to 1, that the truth lies between 44 per cent. and 42 per cent. (Table A 3); but we need examine only 415 things to ensure, with a probability of 9 to 1, that the truth lies between 47 per cent. and 39 per cent. (Table A 5); and only 185 things that, with a probability of 9 to 1, it lies between 49 per cent. and 37 per cent.

If the observed percentage is over 50, we subtract it from 100 and obtain the required figures for the remainder. For example, the figures for observed percentages of 70 per cent., 79 per cent., and 85 per cent. are precisely the same as those for 30 per cent., 21 per cent. and 15 per cent.

On inspecting the tables we see at once that the figures diminish rapidly in successive tables, and also in successive columns (from left to right). From these observations we gather, as we might have expected, that the number of things required to be examined diminishes (*a*) with reduced probability of correctness, and (*b*) with increased statistical error.

The figures increase as we descend the columns. That is, the number of things required to be examined increases as the observed percentage rises from 1 per cent. to 50 per cent.; but after that (by the rule just given) it diminishes as the observed percentage continues to rise from 50 per cent. to 100 per cent.

The tables are calculated for only six degrees of statistical error, namely, from 1 per cent. to 6 per cent. But it is easy to obtain the figures for any required degree, simply by dividing those in the column for 1 per cent. error by the square of the required degree. Thus for a probability of 99,999 to 1, and an observed percentage of 43, we must examine 478 things for a statistical error of 10 per cent., and 4,778,000 things for a statistical error of 1-10th per cent. It will be seen that the columns for 2 per cent., 3 per cent., etc., follow this rule.

It is also easy to obtain approximately the figures for fractional observed percentages, such as 2·5 per cent., or 27·3 per cent., because the increase in the number of things required to be examined is, roughly, proportional to the increase of the observed percentage. Subtract the next lower from the next higher figure in the table; multiply the remainder by the decimal fraction, and add the result to the next lower figure. Thus in Table A 1, in the column for 1 per cent., the figure opposite 2·5 per cent. observed percentage will be about 4,747; and opposite 27·3 per cent. will be about 38,686. But such refinements will rarely be needed.

We are often obliged to examine such a small number of things that the error is evidently greater than the 6 per cent. calculated for in the Tables, and we may wish to know exactly how much it is. In

this case proceed as follows:—Select the degree of probability required, and in the appropriate table look out the observed percentage actually obtained. Take the number opposite to this in the column for 1 per cent. error, and divide it by the number of things actually examined. The error will be the square root of the quotient. Thus, suppose that we have examined only 300 leucocytes, and have found 45 per cent. of these to be mononuclears. The number we ought to examine for a 1 per cent. error at a betting of 9,999 to 1 is 37,459. The ratio of this number to 300 is 125; and the square root of this is 11·2. Hence the statistical error of our work at this betting is 11·2 per cent. But 6,697 leucocytes would have sufficed at a betting of only 9 to 1. The ratio of this to 300 is 22·32; so that the statistical error at 9 to 1 is only 4·7 per cent.—as could have been roughly inferred from Table A 4.

The reader should note the large number of things which must be examined before a result can be obtained to any high degree of probability and within narrow limits of error; and he will doubtless remember many confident assertions based upon much smaller samples.

EXAMPLES

1. How many persons of one nationality must be examined before we can assure ourselves, to a probability of 99,999 to 1, that from 65 per cent. to 67 per cent. of all the nation do not possess blue eyes? *Answer:* 43,744.

2. How many of a patient's leucocytes must be examined before we can bet 999 to 1 that between 42 per cent. and 40 per cent. of all his leucocytes are mononuclear?* *Answer:* 26,194.

3. How many of his leucocytes must be examined before we can bet *about* 100 to 1 that 69 per cent. to 65 per cent. of all his leucocytes are 'polynuclear'? *Answer:* 3,668.

4. How many of his leucocytes must be examined before we can bet 9,999 to 1 that his eosinophile leucocytes number between 1·5 per cent. and 2·5 per cent. of his total leucocytes? *Answer:* 11,868 (Multiply figure in column for 1 per cent. in Table A 2 by 4).

5. On examining 100 of his leucocytes we find that 7 per cent.

* Always supposing that the leucocytes are evenly distributed throughout the circulation.

of them are large mononuclears. How many *more* leucocytes must we examine before betting *about* 10,000 to 1 that the same percentage holds for all the leucocytes in his body within an error of 3 per cent.? *Answer:* 995.

6. On examining 65 case-records we find that 13 of the patients died. How many more case-records must we examine before betting 99 to 1 that the case-mortality of the disease is between 19 per cent. and 21 per cent? *Answer:* 10,551.

7. Our new line of treatment has cured one out of five cases of a hitherto incurable disease. How many more cases must we treat before betting 999 to 1 that we can cure between 15 per cent. and 25 per cent. of all cases? *Answer:* 688.

8. On examining nearly 2,000 of a patient's red corpuscles, we find that 8 per cent. of them are nucleated, and bet 9 to 1 that the percentage of nucleated red-corpuscles in his whole body is—what? *Answer:* Between 7 per cent. and 9 per cent.

9. On examining 220 things, we find that 31 of them belong to a particular class. What is the statistical error at a probability of 99 to 1? *Answer:* 6 per cent.

10. On examining 138 leucocytes we find that 15 per cent. of them are large mononuclears. What is the proportion of large mononuclears in the whole body, at a betting of about 1,000 to 1? *Answer:* Anything between 5 per cent. and 25 per cent. (Find the figure for 10 per cent. error.)

11. On examining 500 malaria parasites, we find that 14 per cent. of them are sexual forms. What is the betting that the statistical error is about 1 per cent.? *Answer:* An even chance.

12. In the same case, what is the betting that the error is not greater than 4 per cent.? *Answer:* 99 to 1.

13. Out of 200 leucocytes we find 23 per cent. to be mononuclears. What is the statistical error at a betting of 999 to 1? *Answer:* 9·79 per cent. (Square root of $\frac{19178}{200}$).

14. Next day, in the same patient, out of the same number of leucocytes, we find 40 per cent. to be mononuclears. What is the statistical error at the same betting? *Answer:* 11·4 per cent.

15. Can we bet 999 to 1 that there has been an increase of mononuclears in this case? *Answer:* No, because the errors overlap;

that is, the difference between 23 and 40 is less than the sum of 9·79 and 11·4.

16. Can we bet 99 to 1 that there has been an increase? *Answer:* Yes, because with this lower degree of probability the sum of the errors, namely 7·7 and 8·9, is less than the difference between the observed percentages, 23 and 40.

17. Working at a probability of 999 to 1, we find that out of 100 leucocytes two are eosinophiles. What is the error? *Answer:* Between 4 per cent. and 5 per cent. What are we to conclude? *Answer:* That we must examine more leucocytes until the error is at least less than the observed percentage.

18. On examining 136 things we find about 10 per cent. to belong to a particular class. What, roughly, is the error at a betting of 9,999 to 1? *Answer:* 10 per cent. (Find the square root of the quotient of 13,621 divided by 136.)

19. On examining 200 things we find 80 of them belong to a particular class. What is the error at a betting of 99,999 to 1? *Answer:* 15·3 per cent. (Divide 46,785 by 200, and find the square root of the quotient.)

20. Working at a probability of 9,999 to 1, and an error of 1 per cent., how many things must we examine in order to assure an observed percentage of 41·7? *Answer:* About 36,789.

1. THE CORRECT PROCEDURE IN PRACTICAL WORK

The Tables will, then, be of practical use in many kinds of sanitary and medical work; as, for instance, in estimating the frequency of death, or of some symptom in a given disease; or of some symptom, such as enlargement of the spleen or rickets, in a population; or in making differential counts of leucocytes in a patient, or of colonies of bacteria growing on a plate culture. But an examination of the Tables will convince us that the procedure now generally adopted in attempting such estimates is very faulty, because observers seldom trouble much regarding that all-important point, the *size* of the sample—that is, the total number of things which they must examine in order to obtain a sufficiently correct result. Certainly, often (though not always), they recognise that the sample must be large; but they usually fix its size quite arbitrarily—as, for instance, when they say beforehand that 200, or 500, or 1,000 leucocytes must be

examined for differential counts. This, however, may lead to the most untrustworthy results, because, as we have seen, the size of the required sample is not fixed, but depends on several factors, including the observed percentage of things of the particular class—that is, the very percentage which we are seeking to ascertain. We cannot, therefore, fix the size of the sample beforehand, but must do so as we proceed in the work.

The size of the sample depends upon three factors, namely:—

(1) The degree of sureness which we have to attain;

(2) The percentage of statistical error, or degree of accuracy, which may be allowed; and

(3) The observed percentage of things of the particular class which we are endeavouring to ascertain.

The *correct procedure* is, therefore, as follows:—

(1) First decide definitely as to the degree of sureness which must be attained, and the percentage of error which may be allowed. These will depend upon the importance of the work and the time which we can devote to it. For strict scientific or large sanitary investigations we may require a very high degree of sureness, say, 99,999 to 1, and very small limits of error, say 1 per cent. And this will be specially the case when we have to compare results obtained at different times; as, for instance, when we wish to know whether the mononuclear leucocytes increase with the progress of a disease (see examples 13-16), or whether an epidemic is diminishing. Here it is absolutely essential that the statistical errors obtained at the two different times are not large enough to overlap. On the other hand, we may often be permitted to adopt lower degrees of sureness and high percentages of error, especially when we are merely seeking some corroborative evidence or when differences between successive estimates are so large and striking that even a large percentage of error cannot mislead the judgment. Here, as in regard to the following paragraph, we must often be guided by the progress of the work. But, as soon as we decide upon these points, we can determine which table, and which column in that table, are to be used.

(2) Secondly, before fixing upon the size of the sample, we should endeavour to obtain by trial a rough estimate of the observed percentage of things of the particular class which we are studying.

Suppose, for example, that we have to make a differential count of leucocytes. Then we do not wish, on the one hand, to allow too much statistical error, or, on the other hand, to waste time over examining too large a sample. Suppose, first, that the 9 to 1 Table is sufficient. Begin by examining 100 leucocytes. Suppose that 40 per cent. of these are mononuclears. Then we can see at once from the Table how many leucocytes must be examined to give a reliable result at that observed percentage. If a 6 per cent. error will suffice, we anticipate that we shall require to examine only 81 more leucocytes. If a 2 per cent. error must be obtained we shall have to examine 1,524 more leucocytes. As we now proceed in the task we shall find that the observed percentage changes considerably when we have examined 200, 300, 400 leucocytes, and so on (we should calculate the percentage, not for each successive batch of 100 leucocytes, but for the total number examined from the beginning). Finally, when about 1,400 leucocytes have been examined, we anticipate that we are approaching the required limit (for 2 per cent. error). Suppose that at 1,559 leucocytes the observed percentage stands at just about 36 per cent.—thus agreeing with the Table. We then stop; having obtained a 36 per cent. ratio, with a betting of 9 to 1, and a statistical error of 2 per cent. That is, the probability is 9 to 1 that the truth lies between 38 per cent and 34 per cent.; and we have not wasted time in reaching this result.

If, however, we require high degrees of probability, or low degrees of error, or both, there will be little use in attempting the preliminary rough estimate by a small sample of only 100 leucocytes, and we had better take for it at once 500 or 1,000 or more as the case might be.

(3) Of course, in differential leucocyte counts we often possess beforehand some inkling of what observed percentage we are to expect. Thus, the eosinophiles are generally few in number, and the 'polynuclears' numerous; and we judge roughly regarding the size of the sample accordingly. The same thing usually happens in other kinds of enquiry.

(4) It is a great mistake to suppose that a large sample will compensate for inaccurate working. These Tables are based on the supposition that each thing examined has been accurately assigned to its proper class. Things which cannot be certainly

assigned to their proper class must be rejected; but the number of them must be noted, and the proportion which they bear to the whole number of things must be afterwards determined, with estimates of probability and error, by precisely the same methods as those described. They constitute, in fact, a third class by themselves.

(5) If the things under study can be divided into three or more classes, determine separately the proportion of each class to the whole. This does not necessarily require different series of investigations. We simply extract the figures from the records; but care must be taken that the samples are sufficient for each class by itself.

(6) If while examining successive samples of things (such as leucocytes) we find that the observed percentage in each sample in succession tends always in one direction, that is, either to increase or to decrease, then we may *suspect* that some influence other than mere chance is at work. The number of successive samples required to verify such a suspicion will depend upon the nature of the material, and might be large; but in many cases if the observed percentage always increases, or always diminishes, in at least five successive samples, then we may have grounds for further enquiry upon the point, or for reference to a trained statistician.

(7) The probability or degree of betting which is generally accepted by statisticians as amounting almost to certainty is 49,999 to 1. The figures for this can be obtained by multiplying the corresponding figures for a betting of 9,999 to 1 (Table A 2) by the factor 1·189; or, what is nearly the same thing, by increasing the figures in Table A 2, by 20 per cent.

(8) Great care must always be taken that samples are chosen really at random, and are not selected with any conscious or subconscious bias.

3. PROPORTIONATELY LARGE SAMPLES

We have hitherto dealt with Comparatively Small Samples—that is, with samples which are small compared with the total number of the things in existence. For instance, if we are studying all the people in a country or all the leucocytes in a patient's body, we shall seldom be able to examine more than a very small proportion of

these people or leucocytes. But there are cases when the total number of things is not so very large that we cannot examine a very considerable proportion of them. Suppose, for instance, that we wish to ascertain the proportion of children with enlarged spleen, not in the whole world, but in a large village; and suppose that there are 200 children in the village, but that we have time to examine only 50 of them. Here the sample is comparatively large, being one quarter of all the children in the village. Or suppose that we can examine 180 of the children; here the sample is so large that it approaches the whole number of things under study (i.e., the children in the village). Obviously, on examining these large samples we shall approach much nearer to the exact truth than would be anticipated from the Tables. In the second case, for instance, we should have to examine only 20 more of the children in order to obtain absolute certainty (provided that the examinations are careful enough). Hence, clearly, the Tables must be corrected for the case of Large Samples. A very suitable and easily applicable method for this purpose is to multiply the statistical error given in the Tables by the Factor.

$$\sqrt{1 - \frac{n-1}{N-1}}$$

where n is the number of things in the sample, and N is the total number of things under study.

Examining this Correction Factor, we see that when n is very small compared with N, the term $\frac{n-1}{N-1}$ becomes so small that it may be neglected; so that the Factor now becomes the square root of unity; that is, unity. This multiplied into the statistical errors given in the Tables does not modify them at all—so that the Tables are then quite correct. Here we have, of course, the case of Comparatively Small Samples, where N is a very large number, such as all the people in a country or all the leucocytes in a person.

Again, if $n=N$, that is, when the sample includes all the things in existence, the term $\frac{n-1}{N-1}$ equals unity, and the Factor becomes zero. This multiplied into the statistical errors makes them vanish. In other words, there is no statistical error because we have reached certainty.

Between these values the Factor is a vulgar fraction, which can

easily be calculated. Thus, if $n = 50$ and $N = 200$, the Factor is the square root of $\frac{150}{199}$; that is, 0·868. This reduces the statistical error, but not much. If $n = 180$, the Factor is the square root of $\frac{20}{199}$; that is, 0·317—which reduces them considerably.

If the total number of things is at all considerable—say over 20—then the Factor becomes nearly the same as the square root of $1 - \frac{n}{N}$. In this case we can give a table of the various values of the Factor which correspond to the various values of $\frac{n}{N}$. In the following Table the proportion of things examined to total things, $\frac{n}{N}$, is given in percentages, and the corresponding values of the Factor are put below:

TABLE B

$100\frac{n}{N}$ =	5	10	15	20	25	30	35	40	45	50
Factor =	0·97	0·95	0·92	0·89	0·87	0·84	0·81	0·77	0·74	0·71
$100\frac{n}{N}$ =	55	60	65	70	75	80	85	90	95	100
Factor =	0·67	0·63	0·59	0·55	0·50	0·45	0·39	0·32	0·22	0·00

Thus, if we examine half the total things, the statistical errors are reduced to about seven-tenths of the values given in Tables A. It will be seen that this Factor makes little difference in the statistical error unless the number of things examined is more than one-tenth of the total.

EXAMPLES

21. Nine out of 145 children in a village are absent. On examining the remainder we find 14 (say 10 per cent.) of them with enlarged spleen. What is the error at a betting of 9,999 to 1? *Answer:* 2·5 per cent. (The Factor is 1/4. See also Example 18.)

22. There are 1,000 people on an island. Out of 100 of these 60 are found to be affected with filariasis. What is the statistical error at a betting of 9 to 1? *Answer:* About 7·7 per cent.

23. Out of 884 people in a town, three-quarters are examined, and 73 of these are found to be in bad health. How many of all the people in the town are likely to be unwell? *Answer:* Between 9 per cent. and 13 per cent., at a betting of 999 to 1.

4. THE NUMBER OF THINGS OF ONE CLASS EXISTING IN A GIVEN AREA, BULK, OR TIME

Suppose that we wish to know the number of things of one kind contained in a given area, bulk, or time. Then the only way to ascertain this with certainty is to count all the things. Suppose, however, that we have no time for this, and must content ourself with counting the things in a measured sample, and then estimating from this observed result the most probable number of the things in the whole area, bulk or time. The question then arises: How large must the sample be in order to reduce the statistical error, with a given degree of probability, to below a given percentage?

For example, suppose that we wish to know how many separate stones there are in a million cubic feet of gravel. We cannot count them all, and must, therefore, content ourself with counting how many there are in a sample of, say, one, two or more cubic feet. In how many cubic feet, then, must we count the separate stones in order to be able to calculate the total with the required degree of accuracy? Or suppose that we wish to know how many leucocytes or parasites there are in the total blood of a patient, then in how much of his blood must we count these objects in order that the most probable truth will lie between sufficiently narrow limits? Shall we take one, two, or more cubic millimetres of his blood for our sample?

First, in order to use the sampling method at all, we must know that the things are equally distributed throughout the area, bulk, or time. If this is not the case, we cannot know that our sample accurately represents the whole material. For example, it would be useless to attempt to estimate the population of Britain by counting all the people in one-tenth of the area of the country because the population is not distributed equally at random everywhere, but is gathered specially into certain districts and cities according to certain economic laws. Similarly, we cannot estimate by taking samples of the peripheral blood how many blood parasites there are in the whole body unless we know that these organisms do not collect specially in certain parts of the circulation.

But—it may be asked—if the things are equably distributed, what further trouble will there be? We have only to count the

number found in any sample, and then to multiply that figure by the total number of samples contained in the whole area, bulk, or time. If the stones are equally distributed in a million cubic feet of gravel, then there will be exactly a million times as many in the whole mass of gravel as there are in one cubic foot. But this is not so. It may be that *by chance* the stones in the first cubic foot taken as a sample are exceptionally large, and therefore are exceptionally few. Or it may happen by chance that the trypanosomes in a first sample of blood are exceptionally numerous, or exceptionally few, as the case may be. We shall then form a totally wrong estimate if we trust merely to the simple but untruthful method just mentioned.

To obtain accurate estimates by any method in cases like these may require the services of a trained statistician, and also, often, a special study of the kind of material under consideration—especially to ascertain whether the things are really equably distributed. But for the purposes of this Article the following Table will often be useful, because it serves to give some idea of the number of things which must actually be counted if they are equably distributed throughout the whole area, bulk, or time.

TABLE C

PROBABILITY	STATISTICAL ERRORS						
	1 %	2 %	3 %	4 %	5 %	10 %	20 %
1 to 1	4,550	1,138	506	285	182	46	11
9 to 1	27,057	6,764	3,006	1,691	1,082	271	68
99 to 1	66,350	16,588	7,372	4,147	2,654	664	166
999 to 1	108,284	27,071	12,032	6,768	4,331	1,083	271
9,999 to 1	151,338	37,835	16,815	9,459	6,053	1,513	378
99,999 to 1	194,938	48,735	21,660	12,184	7,797	1,949	487

Suppose, for example, that a cubic foot of gravel has been found to contain 3,006 stones, then the most probable number of stones in a million cubic feet of the same gravel will be 3,006 millions, within an error of 3 per cent., and at a betting of 9 to 1 ; that is, we may

bet 9 to 1 that the most probable total number of stones in the million cubic feet will lie between about 3,096 and 2,916 millions. Or suppose that 271 trypanosomes have been found in one cubic millimetre of blood in a patient weighing 64·74 kilogrammes (142 lbs., or about 10 stone English), who should contain about 3,000,000 c.mm. of blood altogether; then we may bet 9 to 1 that the most probable number of trypanosomes in the whole of his blood will lie between 894,300,000 and 731,700,000 (10 per cent. error). And if we have counted 4,200 leucocytes in the c.mm. of blood, we may bet 9,999 to 1 that his total blood contains between 13,356 and 11,844 millions of these cells (6 per cent. error).

As stated in Section 1 (page 350) if we wish to know the number of things to be counted in order to yield an error within more than 5 per cent. we have only to divide the figures under the 1 per cent. column twice over by the required percentage (that is, by the square of the required percentage). Thus, for an error of 10 per cent. we divide by 100; and for an error of 31·6 per cent. we divide by 1,000. For example, if we find 27 malaria crescents in 1 c.mm. of the same patient's blood, we may bet 9 to 1 that he contains between about 106 and 56 millions of crescents altogether (always provided that they are equably distributed in the blood).

The above Table is only for *proportionately small samples*, as defined in Section 1 (page 348); that is, for samples which are small compared with the total mass of material. When the sample is more than about one-tenth of the total material we should use the Correction Factor of Section 3 (page 357) for *proportionately large samples*. Thus, if 1,500 leucocytes have been counted in one-quarter c.mm. of blood, and we wish to calculate the number in 1 c.mm., then the error by the above Table is about 10 per cent. at a betting of 9,999 to 1. But by the Table on page 358 the Correction Factor is 0·87 when $\frac{n}{N}$ equals one-quarter, or 25 per cent. Thus the error is not 10 per cent., but 8·7 per cent.; and we may bet 9,999 to 1 that the number of leucocytes in 1 c.mm. of blood is between 6,522 and 5,478. But for the total blood content of 3,000,000 c.mm. the error of 10 per cent. must be maintained, because the one-quarter c.mm. is now a 'proportionately small sample.' This gives the number of leucocytes in the whole body as most probably lying between 19,800 and 16,200 millions.

We can use the Table in another way. Suppose that we have counted 664 things in a sample. Then the error is 10 per cent. on a probability of 99 to 1. Hence we can bet 99 to 1 that all counts in future samples of the same size will lie between 730·4 and 597·6—assuming that the sample is proportionately small. This way of stating the case avoids the necessity of determining exactly the size of the sample compared to the whole material. In blood counts, for instance, we are concerned with the number of things in unit of blood rather than in the whole body, and we often wish to know whether this number is increasing or diminishing. If the number of things in a second sample is outside the limits of error declared from the first sample, we may assume, at the appropriate probability, that there has been an increase or decrease, as the case may be. If otherwise, the difference may be due merely to chance in the taking of the samples, and not to any real change in the total number of things in the whole body.

EXAMPLES

24. We have counted 4,250 red corpuscles in one-thousandth of a cubic millimetre of blood. What is the most probable number in one cubic millimetre, at a betting of 99 to 1? *Answer:* Between about 4,420,000 and 4,080,000.

25. How many may we expect to find in a second sample of the same size, at a betting of 999 to 1? *Answer:* Between about 4,463 and 4,037.

26. A week ago we found 490 red corpuscles in one-ten-thousandth of a c.mm. of a patient's blood. To-day we find only 294 in the same sized sample. May we bet 99,999 to 1 that there has been a decrease? *Answer:* No, the errors overlap. May we bet 9 to 1 that there has been a decrease? *Answer:* Yes.

27. Blood has been diluted 100 times. In $\frac{1}{100}$th of 1 c.mm. of the mixture we found 500 red corpuscles. How many do we expect to find in 1 c.mm. of the blood at a probability of 999 to 1? *Answer:* 5,000,000, with an error of 14·7 per cent.

28. We have found 553 things in a sample. In how many out of 100 similar samples of the same material should we expect to find an error greater than 7 per cent.? *Answer:* In ten.

29. A newly-appointed official finds that he is obliged to write

150 letters during his first week of office. How many letters should he expect, at a betting of 999 to 1, to have to write at the same rate every week in the future? *Answer:* 150, with an error of 26·87 per cent.

30. We have found one filaria embryo in 1 c.mm. of blood. What may we infer regarding the total number in the whole circulation of 3,000,000 c.mm.? *Answer:* The odds are 1 to 1 that the error is less than 67·5 per cent., and that the total number of embryos in the circulation lies between 5,025,000 and 975,000. In one out of two such cases the error may exceed this amount.

II. TABLES

A 1. 99999 to 1

%	Errors					
	1 %	2 %	3 %	4 %	5 %	6 %
1	1930	483	215	121	78	54
2	3821	956	425	239	153	107
3	5673	1419	631	355	227	158
4	7486	1872	832	468	300	208
5	9260	2315	1029	579	371	258
6	10995	2749	1222	688	440	306
7	12691	3173	1410	794	508	353
8	14348	3587	1595	897	574	399
9	15966	3992	1774	998	639	444
10	17545	4387	1950	1097	702	488
11	19085	4772	2121	1193	764	531
12	20586	5147	2288	1287	824	572
13	22048	5512	2450	1378	882	613
14	23471	5868	2608	1467	939	652
15	24855	6214	2762	1554	995	691
16	26200	6550	2912	1638	1048	728
17	27506	6877	3057	1720	1101	765
18	28773	7194	3197	1799	1151	800
19	30000	7500	3334	1875	1200	834
20	31191	7798	3466	1950	1244	867
21	32340	8085	3594	2022	1294	899
22	33451	8363	3717	2091	1339	930
23	34524	8631	3836	2158	1381	959
24	35557	8889	3951	2223	1423	988
25	36551	9138	4062	2285	1463	1016
26	37506	9377	4168	2345	1501	1042
27	38423	9606	4270	2402	1537	1068
28	39300	9825	4367	2457	1572	1092
29	40138	10035	4460	2509	1606	1115
30	40936	10234	4549	2559	1638	1138
31	41698	10425	4634	2607	1668	1159
32	42419	10605	4714	2652	1697	1179
33	43101	10776	4789	2694	1724	1198
34	43744	10936	4861	2734	1750	1216
35	44349	11088	4928	2772	1774	1232
36	44914	11229	4991	2808	1797	1248
37	45440	11360	5049	2840	1818	1263
38	45928	11482	5104	2871	1837	1276
39	46366	11592	5152	2898	1855	1288
40	46785	11697	5199	2925	1872	1300
41	47156	11789	5240	2948	1887	1310
42	47487	11872	5277	2968	1900	1320
43	47780	11945	5309	2987	1912	1328
44	48033	12009	5337	3003	1922	1335
45	48247	12062	5361	3016	1930	1341
46	48423	12106	5381	3027	1937	1346
47	48559	12140	5396	3035	1943	1349
48	48657	12165	5407	3042	1947	1352
49	48715	12179	5413	3045	1949	1354
50	48735	12184	5415	3046	1950	1354

A 2. 9999 to 1

%	Errors					
	1 %	2 %	3 %	4 %	5 %	6 %
1	1499	375	167	94	60	42
2	2967	742	330	186	119	83
3	4404	1101	490	276	177	123
4	5812	1453	646	364	233	162
5	7189	1798	799	450	288	200
6	8536	2134	949	534	342	238
7	9852	2463	1095	616	395	274
8	11139	2785	1238	697	446	310
9	12395	3099	1378	775	496	345
10	13621	3406	1514	852	545	379
11	14816	3704	1647	926	593	412
12	15982	3996	1776	999	640	444
13	17117	4280	1902	1070	685	476
14	18221	4556	2025	1139	729	507
15	19296	4824	2144	1206	772	536
16	20340	5085	2260	1272	814	565
17	21354	5339	2373	1335	855	594
18	22338	5585	2482	1397	894	621
19	23291	5823	2588	1456	932	647
20	24215	6054	2691	1514	969	673
21	25107	6277	2790	1570	1005	698
22	25970	6493	2886	1624	1039	722
23	26802	6701	2978	1676	1073	745
24	27605	6902	3068	1726	1105	767
25	28376	7094	3153	1774	1135	789
26	29118	7280	3236	1820	1165	809
27	29829	7458	3315	1865	1193	829
28	30510	7628	3390	1907	1221	848
29	31160	7790	3463	1948	1247	866
30	31781	7946	3532	1987	1272	883
31	32372	8093	3597	2024	1295	900
32	32932	8233	3660	2059	1317	915
33	33461	8366	3718	2092	1339	930
34	33960	8490	3774	2123	1359	944
35	34430	8608	3826	2152	1378	957
36	34868	8717	3875	2180	1395	969
37	35277	8820	3920	2205	1411	980
38	35655	8914	3962	2229	1427	991
39	36004	9001	4001	2250	1441	1000
40	36322	9081	4036	2270	1453	1009
41	36609	9153	4068	2289	1465	1017
42	36866	9217	4097	2305	1475	1025
43	37093	9274	4122	2319	1484	1031
44	37290	9323	4144	2331	1492	1036
45	37459	9365	4162	2342	1499	1041
46	37593	9399	4177	2350	1504	1045
47	37699	9425	4189	2357	1508	1048
48	37774	9444	4198	2361	1511	1050
49	37820	9455	4203	2364	1513	1051
50	37835	9459	4204	2365	1514	1051

A 3. 999 to 1

%	Errors					
	1 %	2 %	3 %	4 %	5 %	6 %
1	1072	268	120	67	43	30
2	2123	531	236	133	85	59
3	3152	788	351	197	127	88
4	4159	1040	463	260	167	116
5	5144	1286	572	322	206	143
6	6107	1527	679	382	245	170
7	7050	1763	784	441	282	196
8	7970	1993	886	499	319	222
9	8869	2218	986	555	355	247
10	9746	2437	1083	610	390	271
11	10601	2651	1178	663	424	295
12	11435	2859	1271	715	458	318
13	12247	3062	1361	766	490	341
14	13038	3260	1449	815	522	363
15	13807	3452	1535	863	553	384
16	14554	3639	1617	910	583	405
17	15279	3820	1698	955	611	425
18	15983	3996	1776	999	640	444
19	16665	4167	1852	1042	667	463
20	17326	4332	1925	1083	693	482
21	17965	4492	1995	1123	719	499
22	18582	4646	2065	1162	744	517
23	19178	4795	2131	1199	767	533
24	19751	4938	2195	1235	791	549
25	20304	5076	2256	1269	813	564
26	20834	5209	2315	1303	833	579
27	21343	5336	2372	1334	854	593
28	21830	5458	2426	1365	874	607
29	22296	5574	2478	1394	892	620
30	22740	5685	2527	1422	910	632
31	23162	5791	2574	1448	927	644
32	23562	5891	2618	1473	943	655
33	23942	5986	2661	1497	958	666
34	24299	6075	2700	1519	972	675
35	24635	6159	2738	1540	986	685
36	24949	6238	2773	1560	998	694
37	25241	6311	2805	1578	1010	702
38	25512	6378	2835	1595	1021	709
39	25761	6441	2863	1611	1031	716
40	25989	6498	2888	1625	1040	722
41	26194	6549	2911	1638	1048	728
42	26378	6595	2931	1649	1055	733
43	26541	6636	2949	1659	1062	738
44	26682	6671	2965	1668	1068	742
45	26800	6700	2978	1675	1072	745
46	26898	6725	2989	1682	1076	748
47	26974	6744	2997	1686	1079	750
48	27028	6757	3004	1689	1081	751
49	27061	6766	3007	1692	1082	751
50	27071	6768	3008	1692	1083	752

A 4. 99 to 1

%	Errors					
	1 %	2 %	3 %	4 %	5 %	6 %
1	658	165	74	42	27	19
2	1301	325	145	82	52	37
3	1931	483	215	121	78	54
4	2549	638	284	160	102	71
5	3151	788	350	197	126	88
6	3743	936	416	234	150	104
7	4319	1080	480	270	173	120
8	4884	1221	543	306	196	136
9	5434	1359	604	340	218	151
10	5973	1494	664	374	239	166
11	6496	1624	728	406	260	181
12	7007	1752	779	438	281	195
13	7504	1876	834	469	301	209
14	7989	1998	888	500	320	222
15	8461	2116	940	529	339	236
16	8918	2230	991	558	357	248
17	9361	2341	1040	586	375	260
18	9794	2449	1089	613	392	273
19	10211	2553	1135	639	409	284
20	10616	2654	1180	664	425	295
21	11007	2752	1223	688	441	306
22	11386	2847	1265	712	456	317
23	11752	2938	1306	735	470	327
24	12104	3026	1345	757	485	337
25	12441	3111	1383	778	498	346
26	12767	3192	1419	798	511	355
27	13078	3270	1453	818	523	364
28	13376	3344	1487	836	535	372
29	13682	3421	1521	856	548	381
30	13934	3484	1549	871	558	388
31	14193	3549	1577	888	568	395
32	14439	3610	1605	903	578	402
33	14672	3668	1631	917	587	408
34	14890	3723	1655	931	596	414
35	15096	3774	1678	944	604	420
36	15287	3822	1699	956	612	425
37	15467	3867	1719	967	619	430
38	15633	3909	1737	978	625	435
39	15787	3947	1755	987	632	439
40	15925	3982	1770	996	637	443
41	16051	4013	1784	1004	642	446
42	16164	4041	1796	1011	647	449
43	16263	4066	1807	1017	651	452
44	16349	4088	1817	1022	654	455
45	16422	4106	1825	1027	657	457
46	16482	4121	1832	1031	660	458
47	16530	4133	1837	1034	662	460
48	16562	4141	1841	1036	663	461
49	16582	4146	1843	1037	664	461
50	16588	4147	1844	1037	664	461

A 5. 9 to 1

%	Errors					
	1 %	2 %	3 %	4 %	5 %	6 %
1	268	67	30	17	11	8
2	531	133	59	34	22	15
3	788	197	88	50	32	22
4	1039	260	116	65	42	29
5	1286	322	143	81	52	36
6	1526	382	170	96	62	43
7	1762	441	196	111	71	49
8	1992	498	222	125	80	56
9	2216	554	247	139	89	62
10	2436	609	271	153	98	68
11	2649	663	295	166	106	74
12	2858	715	318	179	115	80
13	3061	766	341	192	123	86
14	3258	815	362	204	131	91
15	3450	863	384	216	139	96
16	3637	910	405	228	146	102
17	3818	955	425	239	153	107
18	3994	999	444	250	160	111
19	4164	1041	463	261	167	116
20	4330	1083	482	271	174	121
21	4489	1123	499	281	180	125
22	4643	1161	516	291	186	129
23	4792	1198	533	300	192	134
24	4936	1234	549	309	198	138
25	5074	1269	564	318	203	141
26	5206	1302	579	326	209	145
27	5333	1334	593	334	214	149
28	5455	1364	607	341	219	152
29	5571	1393	619	349	223	155
30	5682	1421	632	356	228	158
31	5788	1447	644	362	232	161
32	5888	1472	655	368	236	164
33	5983	1496	665	374	240	167
34	6072	1518	675	380	243	169
35	6156	1539	684	385	246	171
36	6234	1559	693	390	250	174
37	6307	1577	701	395	253	176
38	6375	1594	709	399	255	178
39	6437	1610	716	403	258	179
40	6494	1624	722	406	260	181
41	6545	1637	728	410	262	182
42	6591	1648	733	412	264	184
43	6632	1658	737	415	266	185
44	6667	1667	741	417	267	186
45	6697	1675	745	419	268	187
46	6721	1681	747	421	269	187
47	6740	1685	749	422	270	188
48	6754	1689	751	423	271	188
49	6762	1691	752	423	271	188
50	6764	1691	752	423	271	188

A 6. 1 to 1

%	Errors					
	1 %	2 %	3 %	4 %	5 %	6 %
1	45	11	5	3	2	1
2	89	22	10	6	4	2
3	132	33	15	8	5	4
4	175	44	19	11	7	5
5	216	54	24	14	9	6
6	257	64	29	16	10	7
7	296	74	33	19	12	8
8	335	84	37	21	13	9
9	373	93	41	23	15	10
10	409	102	45	26	16	11
11	445	111	49	28	18	12
12	480	120	53	30	19	13
13	515	129	57	32	21	14
14	548	137	61	34	22	15
15	580	145	64	36	23	16
16	611	153	68	38	25	17
17	642	160	71	40	26	18
18	671	168	75	42	27	19
19	700	175	78	44	28	19
20	728	182	81	45	29	20
21	755	186	84	47	30	21
22	781	195	87	48	31	22
23	806	201	90	50	32	22
24	830	207	92	52	33	23
25	853	213	95	53	34	24
26	875	219	97	55	35	24
27	897	224	100	56	36	25
28	917	229	102	57	37	25
29	937	234	104	59	37	26
30	955	239	106	60	38	27
31	973	243	108	61	39	27
32	990	247	110	62	40	28
33	1006	251	112	63	40	28
34	1021	255	113	64	41	28
35	1035	259	115	65	41	29
36	1048	262	116	66	41	29
37	1060	265	118	66	42	29
38	1072	268	119	67	42	30
39	1082	271	120	68	43	30
40	1092	273	121	68	43	30
41	1100	275	122	69	44	31
42	1108	277	123	69	44	31
43	1115	279	124	70	44	31
44	1121	280	125	70	45	31
45	1126	281	125	70	45	31
46	1130	282	126	71	45	31
47	1133	283	126	71	45	32
48	1135	284	126	71	45	32
49	1137	284	126	71	45	32
50	1137	284	126	71	45	32

NOTES ON SOME BLOOD PARASITES IN REPTILES

BY

DR. HARALD SEIDELIN

(*Received for publication* 15 *July*, 1911)

I

Filaria (*Mikrofilaria*) *imperatoris* (d'Hérelle and Seidelin, 1909)

Together with F. d'Hérelle I published, in 1909, a short note on two mikrofilariae from the blood of two different snakes from Yucatán, México. We hoped to be able to make further observations on the subject, but so far I have not, for my part, had any opportunity for studying new cases, and probably shall not obtain it soon. I have, therefore, re-examined the specimens from the one case, which belonged to me, and propose now to give a somewhat more detailed description of the parasite. The observation is necessarily incomplete, as I have only had at my disposal dried and stained films, and, moreover, adult filariae were not found. The description and figures may, however, facilitate the identification if some other observer should meet with the same parasite.

The blood films were obtained from a mesenteric artery of a *Boa imperator* which was brought, badly injured but yet with signs of life, to the laboratory. They were fixed in methyl alcohol and stained with Giemsa's stain. As the preparations were only intended to contribute to an investigation of the frequency of haemogregarines they were laid away to be examined later, and thus the opportunity was lost of examining the parasite *in vivo* and of obtaining films for wet fixation.

The parasites were fairly numerous, numbering about seventy in a slide smear. Their general form and structure correspond well to the description and figures of other blood filariae by Annett, Dutton and Elliott (1901), Looss (1905), Manson (1907), and others. The long, slender body has a blunt anterior extremity—the head—and a rapidly tapering tail. The surface of the body shows in some portions a delicate, transverse striation (fig. 1). The head

forms a direct prolongation of the body, and is provided with a fine mouth-opening, situated somewhat to the (ventral) side; besides numerous small reddish dots no other definite structures are observed, especially no 'spicule' or 'prepuce.' Beginning at the neck and continued throughout the body are seen larger granules, which have the aspect and staining properties of cell nuclei. Several hundreds of these granules are present, and they are situated in several longitudinal rows which are, however, interrupted at two or three places. The first interruption seems to correspond to the so-called nerve spot, in which no structure is seen; the second is occupied by the 'excretory cell,' an ovoid body containing a clear space, the outlines of which are nearly concentric with those of the 'cell.' The excretory pore is not seen. The third interruption probably contains the 'genital cell,' but such a body cannot be distinguished. The last few granules in the tail are situated in a single row.

The most peculiar feature of these mikrofilariae is that each embryo is situated in an envelope which is generally egg-shaped, but sometimes more oblong. Only in a few cases is the embryo observed free, but then the empty envelope is seen in its immediate vicinity so that the liberation would seem to have taken place artificially, by the spreading of the blood. The envelopes stain a pale blue, and do not show any structure. In their interior the parasites occupy different positions, being sometimes curled up in a spiral (fig. 2), and sometimes nearly straight (fig. 3); between these two extremes all intermediate positions are seen. One cannot help being impressed with the view that individuals like the one depicted in fig. 2 are embryos enclosed in their egg-membranes. On the other hand, the aspect of individuals like fig. 3 resembles considerably that of an ordinary mikrofilaria in its sheath, although the envelope does not adapt itself to the shape of the worm. As a whole the different forms observed correspond closely to the figures given by Manson (1883), as illustrating the transformation of the egg membrane into a sheath, and it would, therefore, seem that our observation supports the view advocated by that authority upon the process, a view which has not been universally accepted. Such huge bodies as the envelopes would, of course, if comparatively rigid, be unable to circulate through the blood-capillaries of man;

how far they might do so in those of a snake, I do not know, since the blood was, as it has been said, taken from a mesenteric artery, a vessel of a large diameter.

The principal dimensions of the worm and its envelope, as measured on dried and fixed films, are as follow (average of various individuals):—

Total length of body, 167 μ.
Width of body, 4 μ.
Distance from anterior extremity to nerve spot, 45 μ.
Distance from anterior extremity to excretory cell, 65 μ.
Distance from anterior extremity to genital spot, 128 μ.
Diameter of granules, 1·3 μ.
Long diameter of excretory cell, 2·4 μ.
Short diameter of excretory cell, 2 μ.
Length of envelope, 115 μ (maximum 142 μ, minimum 86 μ).
Width of envelope, 34 μ.

In the *Boa imperator* neither adult filariae nor embryos seem to have been observed before, but in the *Boa constrictor* three filariae have been described: *Filaria boae constrictoris*, Leidy, 1850; *F. bispinosa*, Diesing, 1851; and *F. mucronata*, Molin, 1858. They are stated to occur 'between the muscles of the ribs and the integument,' 'in cavo abdominis' and 'in cavitate thoracis and vasa majora' respectively. In no case are embryos mentioned.

Filarial embryos in the blood of other reptiles have been briefly described by Rodhain (1906) and mentioned in a footnote by Prowazek (1907).

REFERENCES

Annett, H. E., Dutton, J. E., and Elliott, J. H. (1901). Report of the Malaria Expedition to Nigeria, II, *Liverpool Sch. Trop. Med. Memoir* IV, pp. 1-92.

Diesing, C. M. (1851). Systema Helminthum II, Vindobonae, p. 278.

d'Hérelle, F. and Seidelin, H. (1909). Sur deux microfilaires du sang des serpents. *C. R. Soc. Biol.*, LXVII, p. 409.

Leidy, J. (1850). Description of three filariae. *Proc. A.N.S.* Phil., pp. 117-118. [Reprinted in Leidy, J. (1904). Researches in helminthology and parasitology, pp. 40-41].

Looss, A. (1905). Filariasis, in Mense's Handbuch der Tropenkrankheiten, I, pp. 157-158.

Manson, P. (1883). The *Filaria sanguinis hominis*, London, p. 25.

Manson, Sir P. (1907). Tropical Diseases, 4th Ed., Lond.

Molin, R. (1858). Prospectus helminthum quae in prodromo faunae helminthologicae Venetiae continentur. Wien, p. 31.

Prowazek, S. v. (1907). Untersuchungen über Haemogregarinen. *Arb. a. d. Kaiserl. Gesundheitsamte*, XXVI, p. 35.

Rodhain, J. (1906). Filaire infectant le sang chez l'Agama Colonorum dans l'Ubangi. *Centralblatt f. Bakt. u. Parasitenk.*, XLII, pp. 545-546.

II

Haemogregarina imperatoris

In the same blood-smears a haemogregarine was observed which probably represents a new species, since it shows some peculiar morphological features, and, moreover, differs from most other known haemogregarines by the deleterious influence which it exerts upon its host-cells.

The infection was an abundant one, and the elements observed belong evidently to different phases of evolution; but the scarcity of the material at my disposal precludes a full consideration of these questions.

The intracorpuscular parasites which appear least advanced in development are nearly cylindrical bodies with slightly rounded extremities; their length is somewhat less than that of a normal erythrocyte. The protoplasm stains very feebly, and contains only a few reddish granules (Giemsa stain) or no granules at all. The nucleus is generally situated in the central portion of the body, and shows a dense chromatin reticulum, but no definite structural elements can be distinguished. A capsule seems always to enclose the parasites, but it is not always well defined (fig. 16).

In the somewhat larger forms, the granules in the protoplasm have increased both in number and size and, when prominent, stain exactly like chromatin (figs. 17 and 18). The granules show a more or less irregular distribution, and two or three of them may be exceptionally large (fig. 19), but no one can be pointed out with certainty as blepharoplast or other definite structure. Besides the large granules others may be present which are fine, dust-like, and occasionally very numerous. Granules are as a rule not mentioned in the description of haemogregarines, but those here described evidently correspond to the volutin-granules of Reichenow (1910). The protoplasm shows, moreover, in the advanced stages, a diffuse colouring, pale blue or sometimes more pink. The nucleus is similar to that of the smaller forms, but it is more often eccentrically situated, and there can frequently be seen one or two nucleoli in its interior (fig. 20). The chromatin of the nucleus sometimes forms well delimited large granules, which are united only by delicate filaments (fig. 21), and in a few cases a formation

of definite chromosomes seems to have taken place, eight such being distributed in two rows of four each (fig. 22); some more advanced stages of division are also observed (fig. 23). The capsule is in the larger forms fairly well defined, and its extremities are often delimited from the central portion by delicate lines which are stained red (figs. 17 and 25); such lines are considered constant by Sambon (1908-9), but they are seldom mentioned or figured by other authors. I have seen them frequently in other haemogregarines also, but by no means constantly, and never so sharply defined as they appear in Sambon's somewhat schematic figures. If this author is right in describing them as lines of cleavage, it is readily understood that they may be inconstant.

In their most advanced stages the intracorpuscular elements attain almost the dimensions of a normal erythrocyte (figs. 24 and 25). They still conform essentially to the description already given, but in some individuals one extremity is very slender and bent against the body in an acute angle (fig. 26).

A number of parasites belong to a different type. They are long and slender, with one blunt extremity, and the other slowly tapering. Being doubled up they easily find room in the erythrocytes, although they are much longer than their host-cells; sometimes the extremity of the tail is again bent in a sharp angle against the body. The protoplasm stains dark blue or violet, and contains a number of fine and occasionally a few coarse granules. The nucleus is situated near the bend, in the anterior half of the body; it is approximately quadrangular, and occupies the whole of the width of the body. It is sharply limited and deeply stained (fig. 27). The long forms are in this case always intracorpuscular; in the blood of other snakes, however, I have repeatedly seen them free in preparations which were taken from the living animal and immediately fixed. I emphasize this, because Flu (1910), Reichenow (1910), and others state that these elements are always intracorpuscular, normally, and become free only under abnormal conditions, as when the blood is being preserved outside the body.

These long elements have, according to Lutz (1901), and most other authors, no direct connection with the more oval forms; the former are derived from mikrozoits, the latter from makrozoits.

Reichenow (1910), however, asserts that the long forms are at a later stage of the evolution transformed into oval bodies, the extremities being drawn in when the parasites are preparing to divide. All schizonts are said to undergo such a transformation, and it would seem not at all unlikely that some of our larger forms belong to this group, the significance, especially of the large oval elements with short slender tail (fig. 26), becoming intelligible if they are considered as transitional stages. It is, of course, possible to adopt this view with regard to the larger forms only; the small oval parasites must have a different origin, and would, according to Reichenow, represent gametes, but this view does not agree with the presence of mitotic figures in several of them. The distinction between male, female and indifferent haemogregarines which is made by many authors depends, however, to a large extent on analogy, and cannot be too carefully considered before being accepted. In the present case no such differentiation can with certainty be made.

In this case no small parasites were seen free in the blood-plasma, except the one depicted in fig. 28, which appears to have just escaped from its host-cell. An erythrocyte is also shown in fig. 29, which evidently has harboured a parasite. The possibility cannot be denied that these appearances may have been produced artificially. Another very interesting individual is seen in fig. 30; it consists of a large body with faintly blue-stained protoplasm and numerous chromatin-granules, whilst no well defined nucleus is seen. At one extremity of this body is seen another structure, much smaller and very slender, half inside, half outside the larger one; the smaller element is wholly chromatin-stained. The whole appearance suggests strongly the penetration of a mikrogamete into a makrogamete. The possibility of conjugation taking place in the body of the snake is admitted by Hartmann and Chagas (1910). So far very little is known about the sexual phase of the snake-haemogregarines and about the allied subject of their transmission; with regard to *Haemogregarina stepanowi* of tortoises, Reichenow (1910) has shown that the sexual phase is found in a leech.

A few words should now be said about the effect of the haemogregarines on the erythrocytes. In the case of the smaller

parasites their host-cells do not show any important alterations, excepting a slight increase in size and a dislocation of the nucleus. Corresponding to the somewhat larger forms, the erythrocytes are not only enlarged, but often differentiated into a pale peripheral portion and a more deeply staining zone surrounding the parasite. The corpuscles which harbour the most advanced stages of parasites are of enormous size, their length being about twice the normal; they have apparently lost all their haemoglobin, and have become mere shadows, the outlines of which it may be difficult to distinguish. In a few instances numerous fine red granules are seen in the decolourized erythrocytes, perhaps representing remains of the broken-up haemoglobin, but closely resembling the fine granules of the parasites (fig. 25). The nuclei become disfigured by compression and their structure more dense, but no fragmentation is observed, as has been described by Marceau (1901) and others. This deleterious influence on the erythrocytes constitutes a considerable difference from what is the case in most haemogregarine-infections, in which as a rule no alteration of the host-cells is observed, or (in the case of *Karyolysus* sp.) only of the nucleus. Prowazek (1907), however, gives figures (from lizards) very similar to our own.

Besides these parasites I must briefly mention other bodies which are of interest, as they may easily be mistaken for free forms of haemogregarines. In fact, I am not absolutely sure that they are normal blood elements, but there is a great probability in favour of their being so. I refer to the bodies depicted in figs. 4-11 on Plate XIV, which are characterized by a large, well-staining nucleus, a nearly unstained protoplasm, sometimes very scarce, and a well-defined red border-line. Two nuclei may be present, as in fig. 11, but no mitotic figures are seen. These corpuscles are similar to haemogregarine-merozoites, as figured by Reichenow (1910) and others, but on the other hand they bear a striking resemblance to some figures which are given by Werzberg (1910). This author has found such bodies in the blood of *Tropidonotus natrix*, and in *Chamaeleon* sp., and is inclined to consider them as thrombocytes, though he leaves room open for doubt. On examining, here in England, the blood of a *Tropidonotus natrix*, I have also found similar cells, and this

circumstance, of course, confirms the identity of those seen by Werzberg and by myself. One considerable difference seems, however, to exist, namely, in the staining of the nucleus. The figures of Werzberg show a very pale nucleus, whilst my preparations, both from *Tr. natrix* and especially from *Boa imperator*, show a deep staining of the nucleus. This may be due, perhaps, to the different methods of staining, Werzberg having used Pappenheim's so-called Unna-Ziehl stain, and I the Giemsa stain; but as other nuclei, especially of the erythrocytes, in Werzberg's figures are deeply coloured, the explanation is not quite satisfactory. However, it may be admitted that the bodies are probably of the same nature, and are constituents of the blood; this is so much the more likely, as, in *Tr. natrix* they do not show any movements in fresh preparations, as haemogregarines probably would do. It then remains to consider what their real nature may be. In both cases the border lines stain red; this seems by the Unna-Ziehl stain to indicate a relationship to the erythrocytes, as the cytoplasm of these elements takes the same colour. But in my specimens the red is not like the eosin colour of the erythrocytes, it resembles much more the azur-eosin stained chromatin. By other methods, it does not give the reaction of either. It does not stain like chromatin with iron-haematein, nor does it take up eosin, like haemoglobin; in both cases it remains unstained. Moreover, it retains its colour badly; in Giemsa-preparations it is only visible in dry smears when no differentiation is employed. Subsequent to the differentiation with acetone which becomes necessary when the 'wet method' is used, no red colour is to be found. By this differentiation the nuclei of these bodies are also often to a large extent decolourized, though all the surrounding erythrocyte-nuclei are deeply stained. In fresh preparations it is in most cases difficult to observe more than the nuclei of these cells, but occasionally the border-line appears quite sharp, and the protoplasm very slightly granular. Moreover, their protoplasm appears to be very fragile, or at least very plastic, since the bodies in the stained preparation present very irregular and varied forms. The border-line is evidently but loosely connected with the cytoplasm, since it often becomes separated from its periphery and takes on different positions, sometimes so peculiar that a certain resemblance to a

flagellum is produced (figs. 4, 7 and 8). How far these characters are artificially produced by the preparation of the films it is difficult to say, since an exact observation of the bodies was not possible in the specimens which were examined in the fresh state or treated by 'wet' fixation and staining, as above-mentioned.

Similar bodies, but without a definite border-line, were found in the blood of other snakes in Yucatán; haemogregarines were also present. Only in the case of the *Tropidonotus natrix* have no intracorpuscular parasites been detected. No relationship is apparent to other blood elements; at present these bodies must be classified as 'thrombocytes,' a special kind of cells, probably corresponding to the platelets of higher animals.

Another peculiar kind of cell, the significance of which has not been determined, is represented in figure 12. They give at first view the impression of leucocytes, but a more close inspection shows them to be enclosed in a large, faintly eosin-stained protoplasmic body, similar to that of an altered erythrocyte. Their outlines are nearly circular, they have a granular, dark-staining protoplasm, and an eccentrically situated dense nucleus. It cannot be determined if these bodies are of a parasitic nature, or if they represent a stage of evolution of certain blood-elements.

REFERENCES

Fiu, P. C. (1910). Über Haemogregarinen im Blute Surinamischer Schlangen. *Arch. f. Protistenk.*, XVIII, pp. 190-206.

Hartmann, M., and Chagas, C. (1910). Vorläufige Mitteilung über Untersuchungen an Schlangenhaemogregarinen. *Arch. f. Protistenk.*, XX, pp. 351-360.

Lutz, A. (1901). Über die Drepanidien der Schlangen. *Centralbl. f. Bakt. u. Parasitenk.*, Abt. I, XXIX, p. 390.

Marceau. (1901). Note sur le *Karyolysus lacertarum*. *Arch. de Parasitol.*, IV, pp. 135-142.

Prowazek, S. v. (1907). Untersuchungen über Haemogregarinen. *Arb. a. d. Kaiserl. Gsndhtsamte.*, XXVI, pp. 32-36.

Reichenow, E. (1910). *Haemogregarina stepanowi*. *Arch. f. Protistenk.*, XX, pp. 251-350.

Sambon, L. W. (1908-9). Haemogregarines of Snakes. *Journ. Trop. Med. and Hyg.*, XI, pp. 355 and 374, XII, pp. 22, 38 and 70.

Werzberg, A. (1910). Über Blutplättchen und Trombozyten. *Fol. haematol.*, X, pp. 301-390 (p. 368).

III

Amoeboid parasites in the blood-plasma of a Lizard

In the blood of a lizard (*Lacerta* sp.) from Yucatán, which has not been identified, I observed amoeboid parasites in the plasma, while no intracorpuscular forms were seen. Similar known parasites are, at least in some stages of their development, intracorpuscular. Individuals of very different sizes and shapes were observed but all seem to belong to the same kind of organism. The differences correspond probably to different evolutionary phases, although of course the results cannot be entirely relied upon to show exact details considering the technique employed (dry smears, methylalcohol fixation, Giemsa stain). I shall consequently not attempt a detailed description, much less classification, but only shortly state with the aid of illustrations what I saw.

A large number of minute bodies are seen with a fine chromatin dot and a blue protoplasm which is occasionally solid, but as a rule forms a more or less delicate ring around a comparatively large vacuole (fig. 31).

From this stage all subsequent gradations in size are met with (figs. 32-40) until the largest forms which consist of a protoplasmic body the size of which approximates to that of an erythrocyte, and which shows a granular structure and contains numerous small vacuoles and very often one or two larger ones. In most cases one nucleus is present; it is always small and consists of a central darker, and a peripheral less intensely stained portion; often a second, still smaller chromatin-stained element is also present at a considerable distance from the nucleus (fig. 36), and occasionally two nuclei of about equal size are seen (fig. 33); when the latter is the case the cytoplasm shows a certain symmetry and we possibly have to do with a divisional phenomenon. This would also agree with the regular, rounded shape of such elements, but, on the other hand, they are not of particularly large dimensions. In several instances no definite nucleus is present, but several small chromatin-granules. Many of the parasites show pseudopodia in different numbers and of different shapes. The fixation has probably given them a more rigid aspect than they would have had if examined in the living state.

Forms like those shown in figs. 41 and 42 may possibly be distorted amoebae, although the possibility of different kinds of parasites being present must also be considered.

It is remarkable that this extracorpuscular parasite appears to have a more pronounced effect upon the erythrocytes than intracorpuscular parasites often have. Many erythrocytes are metachromatic and vacuolated, and in places where they are closely grouped together the plasma shows a faint eosin staining, a sign of haemolysis having taken place. Related to this phenomenon is probably another which was also observed in this case, namely, the presence of numerous pigmented leucocytes, as shown in the figs. 13, 14 and 15. I can find no mention of these in works on haemogregarines or other intracorpuscular parasites of reptiles. Nor have I observed them in such infections; besides in the case here reported I have only met with pigmented leucocytes once in a lizard, but I have no notes on the presence of parasites in that case. The aspect of the pigment-granules resembles that of malarial pigment, but no statement as to its nature can be made. One would naturally be inclined to connect the pigment with the destruction of haemoglobin; it may be pointed out, however, that Downey (1910), who recently has mentioned the existence of similar pigment in phagocytes from the lympho-renal tissue of a fish (*Polyodon spathula*) describes in detail the transformation, not of the cytoplasm of the erythrocytes, but of their nuclei, into pigment. Here is evidently a point which, for general pathological reasons, deserves further investigation.

REFERENCE

Downey, H. (1910). Phagocytosis of Erythrocytes, etc. *Fol. haematol.*, IX, pp. 81-86.

DESCRIPTION OF PLATES

All figures have been drawn with Abbé's apparatus, using Zeiss's 3 mm. apochromatic objective and, in the figs. 4-42, compensating ocular 12; for fig. 1 compensating ocular 8, and for figs. 2 and 3 compensating ocular 4 has been used. The magnifications are respectively 1300, 850 and 450 linear. Most of the figures have been executed by Mrs. Margrethe Seidelin.

PLATE XIV

Figs. 1-3. Filaria-embryos.

Figs. 4-11. Various forms of 'Thrombocytes.'

Fig. 12. Leucocyte-resembling cell in large protoplasmic body; to the left a lymphocyte.
Figs. 1-12 from the blood of *Boa imperator*.

Fig. 13. Pigmented mononuclear leucocyte.

Fig. 14. Pigmented leucocyte with two nuclei.

Fig. 15. Pigmented and unpigmented leucocyte fusing together
Figs. 13-15 from the blood of *Lacerta* sp.

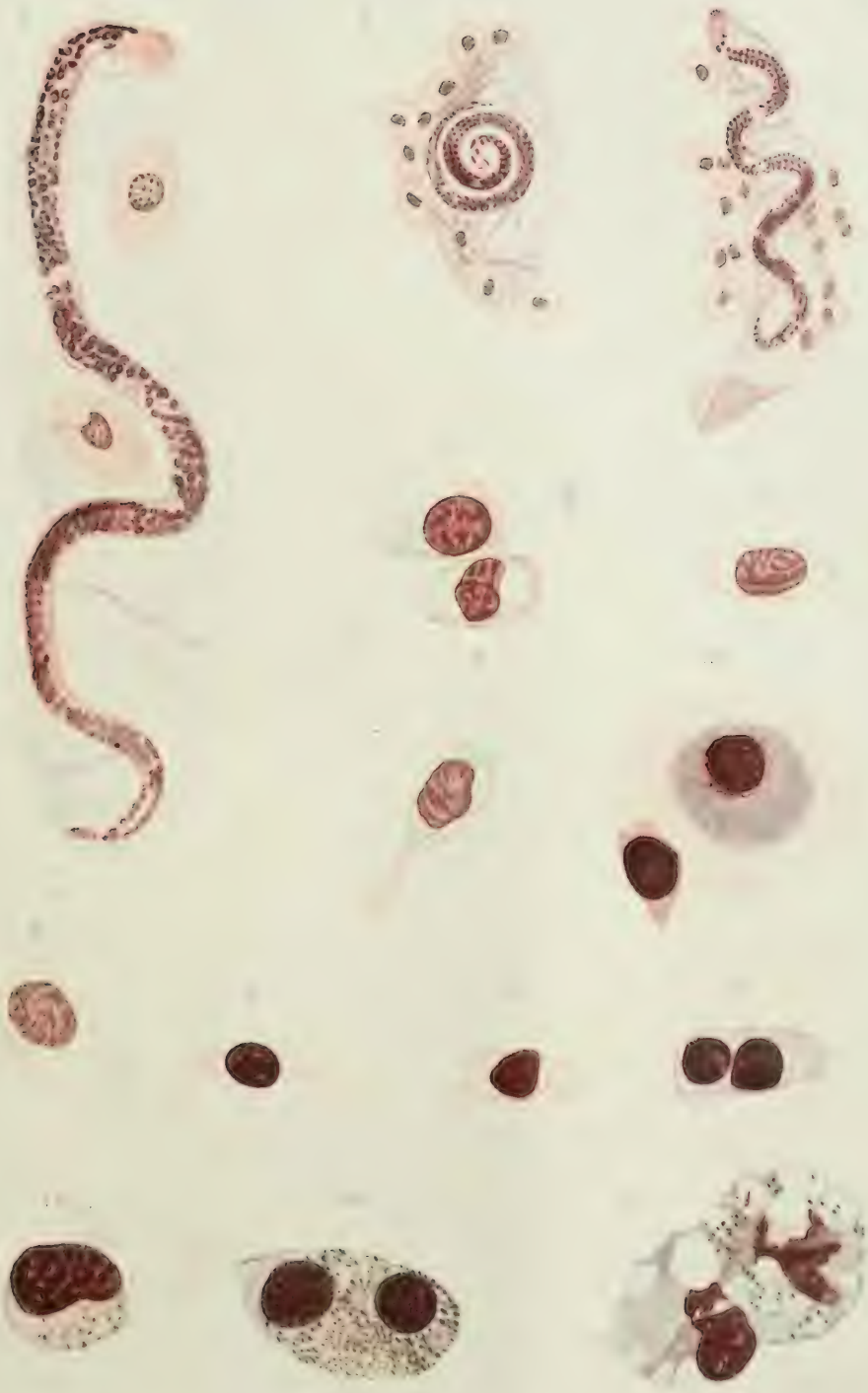


Plate XV

Figs. 16-30. Haemogregarines in blood of *Boa imperator*.

Figs. 16 and 17. Young forms with pale protoplasm and few granules.

Figs. 18-20. Larger forms with more deeply stained protoplasm which contain numerous large granules; in fig. 18 three such granules are very prominent.

Fig. 21. The chromatin in the nucleus forms regular granules.

Figs. 22 and 23. Mitotic figures.

Figs. 24 and 25. Large forms in enlarged and decolourized erythrocytes; in fig. 25 and 26, especially in the first, numerous fine red granules are seen in the cytoplasm of the erythrocyte.

Fig. 27. Long, slender form.

Fig. 28. Parasite escaping from host-cell.

Fig. 29. Empty host-cell.

Fig. 30. Large free form; conjugation ?
In Figs. 19, 20, 25 and 26 cleavage lines are seen.

Figs. 31-42. Parasites from the blood of *Lacerta* sp.
In Figs. 35, 39 and 40 pseudopodia are seen. Figs. 41 and 42 distorted amoebae or flagellata.

Plate XV.



SOME EXPERIMENTS ON LARVICIDES

BY

SIR R. ROSS, K.C.B., F.R.S.,

AND

E. S. EDIE, M.A., B.Sc.

(*Received for publication 17 July*, 1911)

At present the substance in most general use as a larvicide is petroleum, which acts by forming a thin film on the surface of the water, thereby drowning the larvae when they rise to the surface to breathe. In the case of enclosed bodies of water, such as wells and tanks, this usually acts admirably at first, but in the course of a short time the film, unless very thick, gets broken. This happens much more readily in pools or stagnant places on river banks where mosquitos breed. The film of petroleum in these cases soon gets broken or blown to one side sufficiently to allow the mosquitos to lay their eggs, and the larvae to develop into mosquitos. Moreover, the presence of grass or weeds on the banks of these pools prevents the formation of an unbroken film over the whole surface, and this permits the reproductive processes to go on more or less uninterruptedly.

Petroleum has other disadvantages arising from its inflammability and its liquid nature, which render it less convenient for transit.

An ideal larvicide would appear to be a solid substance which kills larvae even when used in the form of an extremely dilute solution. This would be convenient for use, as no great weight of material would have to be carried from place to place. The solution, when diluted to the maximum point at which it will still kill larvae in a reasonable time must also be harmless to men and domestic animals if the water is liable to be used for drinking purposes. Should there be no chance of the water being drunk, however, a stronger solution may be used, and the larvae killed more quickly.

We have tried a number of solutions as larvicides, and an

account of the results obtained with those substances which we thought might be most suitable is given in the following pages. Most of our experiments were carried out with the larvae of *Culex pipiens*, about fifty larvae being used in each case.

We first tried the effect of a larvicide recommended by Le Prince, quoted by Ross (1910), prepared by dissolving resin in crude carbolic acid, and treating the solution with caustic soda. We found it difficult to obtain a proper emulsion without sediment, however, as the resin did not dissolve readily in the carbolic acid. This larvicide has been used with success in America, where it is said to cost only sevenpence a gallon, the average mixture containing 300 gallons crude carbolic acid, 200 pounds resin, 30 pounds caustic soda.

Our results with this larvicide are as follows:—

One part larvicide in 500 parts of water killed 80 per cent. of the larvae (*Culex pipiens*) in two hours, and the remainder in three and a half hours.

One part larvicide in 1,000 parts of water killed 70 per cent. of the larvae in four hours, and most of the remainder by next morning (eighteen hours). One larva, however, lived in this solution for twenty-two hours.

One part larvicide in 2,000 parts of water killed about 30 per cent. of the larvae in six hours, but the rest of the larvae were killed very gradually, some surviving for about fifty hours.

We used the different ingredients in the proportions mentioned above in the preparation of this larvicide, the value of which seems to depend very largely upon the composition of the crude carbolic acid used. Le Prince found this larvicide to act more quickly and at a greater dilution than we did, perhaps because he employed a stronger carbolic acid from which to make the final product.

We next tried an emulsion prepared by the 'Sanitas' Company, Limited, called 'Sanitas-Okol.' This appears to contain a large proportion of phenols and allied compounds, and when much diluted mixes well with water.

We obtained the following results with 'Sanitas-Okol,' using *Culex pipiens* larvae.

One part 'Sanitas-Okol' to 600 parts of water killed all the larvae in fifteen minutes.

One part 'Sanitas-Okol' to 1,000 parts of water killed about 40 per cent. of the larvae in fifteen minutes, and the rest in seventy-five minutes. Many of the pupae were nearly ready to hatch out into mosquitos, at which stage we frequently found them to be more resistant to the action of larvicides, perhaps because they are then less firmly attached to the outer shell, which prevents the solution reaching their tissues so readily.

One part 'Sanitas-Okol' in 2,000 parts of water killed 50 per cent. of a young lot of larvae in sixteen minutes, and the rest in twenty-eight minutes.

One part 'Sanitas-Okol' in 2,500 parts of water killed all the larvae except one in seventy minutes, the last being dead in ninety minutes.

One part of 'Sanitas-Okol' in 5,000 of water killed 40 per cent. of the larvae in seventy minutes, and the rest in two and a half hours.

In another experiment this dilution (one in 5,000) killed 50 per cent. of the larvae in one and three-quarter hours, and all but three in three and a half hours, the last three being dead in about five hours.

One part 'Sanitas-Okol' in 10,000 parts of water killed all the larvae except two in six hours, the last two dying in the course of the night.

With one part 'Sanitas-Okol' in 20,000 parts of water about 10 per cent. of the larvae lived for twenty-one hours, while two larvae were still alive after another twenty-four hours.

We have also tried the effect of 'Sanitas-Okol' on the larvae of *Anopheles bifurcatus*, and found as follows:—

One part 'Sanitas-Okol' to 5,000 parts of water killed eighteen larvae out of twenty in two and a quarter hours, the other two living for three and a quarter hours.

One part 'Sanitas-Okol' in 10,000 parts of water killed twenty-three larvae out of twenty-six in five and a half hours, and the rest in about eight and a half hours.

It will be seen that 'Sanitas-Okol' acts very powerfully as a larvicide, and can be used quite satisfactorily in dilutions up to 1 in 10,000. It is also quite non-poisonous at this great dilution.

In view of the fact that mercuric chloride acts so well as a

germicide, we thought it well to try the effect of solutions of this compound on Culex larvae.

One part mercuric chloride in 2,000 parts of water only killed the larvae extremely slowly, one larva living for three days.

One part mercuric chloride in 1,000 parts of water killed all the larvae except one in eighteen hours.

We carried out no further experiments with mercuric chloride, as it is evidently unsuitable as a larvicide from its poisonous nature and comparatively feeble action on larvae. It would also be too expensive to use on a large scale.

The same objections can be taken to the use of copper sulphate, which failed to kill all the larvae in two days, even when used in the form of a solution containing 1 part of copper sulphate to 500 parts of water. A solution of cupric hydrate in ammonia, containing the same proportion of copper as mentioned above, had no better effect as a larvicide, although one part of ammonia solution (34 per cent.) to 125 parts of water killed all the larvae in twenty minutes.

Oxalic acid was not found to act powerfully on Culex larvae. A solution containing one part of the acid in 1,000 parts of water killed the larvae slowly, one surviving eighteen hours, and when one part of oxalic acid in 2,000 parts of water was used 10 per cent. of the larvae lived for about thirty hours.

It may be of interest to add that saponin (one part to 400 parts of water) was found to have no effect on larvae. While we had scarcely considered it in the light of a possible larvicide, we thought it worth while to try the effect of keeping larvae in such a solution, in view of the haemolytic action of saponin on the blood of higher animals.

We have finally turned our attention to potassium cyanide as a larvicide. As this substance is very much more poisonous than any others we had employed we did not use solutions as dilute as one in 1,000, which would be unsafe should there be any possibility of any water thus treated being drunk.

The following results were obtained with potassium cyanide, using *Culex pipiens*.

One part cyanide in 26,000 of water killed all the larvae in less than two hours.

One part cyanide in 58,000 of water killed all the larvae in three and one-third hours.

One part cyanide in 106,000 parts of water killed all the larvae in five hours.

In another experiment with nearly the same strength (one in 110,000), all the larvae except two were killed in five hours, the last two living about seven and a half hours.

One part cyanide in 240,000 parts of water killed all the larvae in the course of a night (less than sixteen hours from the time the cyanide was added).

One part cyanide in 303,000 parts of water killed about 50 per cent. of the larvae in six and a half hours, and all except one in twenty-two hours.

We have not carried out any experiments with weaker solutions of potassium cyanide than last mentioned, as we did not consider it advisable to use solutions in which larvae can live for a longer period than about eighteen hours, and a solution of one part cyanide to 300,000 parts of water was found by repeated experiment always to satisfy this condition, though in some cases individual larvae survived about twenty-four hours.

We have tried the effect of a solution containing one part potassium cyanide to 300,000 parts of water on two lots of Anopheles larvae, and in both cases about 80 per cent. of the larvae were killed in less than eight hours, and the rest in from twelve to fifteen hours.

Strychnine in dilutions greater than one in 50,000 parts of water appeared to have no effect on larvae. As we had obtained much more satisfactory results with potassium cyanide we did not try solutions of strychnine stronger than the above.

We have been unable to discover any other substance whose potency as a larvicide approaches that of potassium cyanide. This compound also possesses the advantage of being easily carried about and of being comparatively cheap. Its one drawback lies in the fact that it is extremely poisonous, and it is apt to undergo partial hydrolysis with the production of a certain proportion of free prussic acid.

On the other hand the use of potassium cyanide can be restricted to stagnant water which is not used for drinking purposes, while

it must be remembered that water containing cyanide in the proportion of one to 250,000 or 300,000 would have to be consumed in very large quantities before having any deleterious effects.

We have endeavoured to find the most convenient form in which to use potassium cyanide as a larvicide. It is, of course, most conveniently carried about in the solid form, but if a piece be simply thrown into a pool of water it sinks, and although it is very readily dissolved the solution may not diffuse rapidly through the whole pool. Accordingly, we tried mixing the cyanide with a floating soap, both in the form of powder, and compressing the mixture into pills or tablets. These will still float, provided that the proportion of cyanide to soap is not too high, and that too great pressure is not used in the preparation of the tablets. In the latter case the resulting tablets are too dense, owing to there being but little air retained in the substance, and consequently they sink. The advantage of using these floating tablets is that the cyanide soon dissolves out, and the solution being heavier than water diffuses comparatively rapidly through the whole mass of the water, and thus practically none of the larvae escape exposure to the action of the drug. Other substances of a light and porous nature may, of course, be used instead of soap as a medium for the preparation of floating tablets containing the requisite amount of cyanide. These substances should preferably be insoluble, or at least of a comparatively inert nature.

A convenient size of tablet is one containing three or four grains of cyanide, which will suffice for twelve or sixteen gallons of water. These should be sealed up in tins of about 100 tablets, and, of course, all precautions must be taken to prevent any danger of tablets being eaten by children or others. Instructions ought also to be given that no water which is to be used for drinking purposes is to be treated with cyanide. With these precautions there is practically no danger of any harm resulting from the use of potassium cyanide in this form as a larvicide. It is unnecessary to refer here to previous experiments on larvicides, as these are familiar to all students of the subject.

REFERENCE

Ross, Sir R. Prevention of Malaria (John Murray), section 43.

AN INVESTIGATION OF THE EFFECTS PRODUCED UPON THE EXCRETION OF URINARY PIGMENTS BY SALTS OF QUININE

BY

W. M. GRAHAM, M.B.

(DIRECTOR, MEDICAL RESEARCH INSTITUTE, LAGOS, S. N.)

(*Received for publication* 27 *June*, 1911)

In view of the preponderating rôle attributed to salts of quinine in the production of blackwater fever, it has been evident to me for some time that useful information might be secured by examining the action exerted by these salts upon the blood of a healthy adult, living in a malarial country, who had become accustomed to their prophylactic use. It was possible that the action which these salts exerted upon the healthy differed only in the amount of the reaction from that which they exert in a case of disease. In the healthy, the action exerted would be more easily observed in the absence of the various complications introduced into such an investigation by an attack of malarial fever.

Then, the fact that the effect produced upon the urinary pigments by a prophylactic dose of quinine might be used as an index to that exerted on the blood suggested itself, and an examination of the effect of a single dose of the drug was undertaken.

The subject of the experiment was a healthy European adult, long resident in West Africa, where, on several occasions in previous years, he had suffered from attacks of malarial fever (malignant tertian).

The investigation was conducted as follows:—All urine passed between 9.30 p.m. (bedtime) and 7.30 a.m. (breakfast) was collected in a clean beaker for examination. This period of ten hours was selected because of its convenience, and because during this period the environmental conditions would remain fairly

uniform; for no food or fluids would be taken, the temperature in bed would be nearly uniform, and the amount of sweating would be less subject to alteration by changes in temperature, amount of exercise, amount of clothing, or from leaving the shade of the house for the higher temperature out of doors.

An attempt was also made to keep the dietary as uniform as possible during the period of experiment. No fluids were taken except at meals, and then in equal measured amounts. The bowels were naturally opened twice a day, but there was no diarrhoea. The occupation was sedentary, a short walk being taken daily at sundown.

On the first day of the experiment all urine passed between 9.30 p.m. and 7.30 a.m. was collected to serve as a control, representing the normal excretion uninfluenced by the action of a salt of quinine. On the following night, at 9.30 p.m., a dose of fifteen grains of quinine hydrochloride (B. W. & Co.'s tabloids) was taken with six ounces of water, a similar amount of water having been taken on the first night and on all the subsequent nights of the experiment. All urine passed in the ten hours from 9.30 p.m. to 7.30 a.m. on the seven subsequent days was collected and a preliminary examination of the amount, colour, specific gravity, and reaction, was made.

It was then prepared for the photographing of its absorption spectrum in the following manner. The urine collected each morning was poured into a tall measuring cylinder, covered and placed before a well-lighted window for two hours, so as to ensure the complete conversion of urobilinogen to urobilin. Then 100 c.c. of this urine was placed in a beaker and rendered alkaline by the addition of a measured amount of ammonium hydrate (NH_4OH). The precipitated phosphates were removed by filtration, and to the clear filtrate a solution of zinc chloride ($ZnCl_2$) was added cautiously until the precipitate formed began to remain undissolved. The urine was then again filtered, poured into a Baly tube and at once photographed in a Hilger quartz spectrograph by acetylene gas light projected through a quartz condenser. By the use of a Baly tube it was possible to produce readily on the same plate a series of ten photographs taken through regularly diminishing depths of urine under such uniform

conditions that the amount of absorption shown by the different depths could be compared; and on different days, when dealing with urine of varying specific gravity, a urine of low specific gravity could be compared with one of high specific gravity without the necessity of diluting the latter with water, a device likely to introduce a variable error.

The photographs were taken upon the Wratton and Wainwright's pan-chromatic plate. This plate, though the best procurable, is, unfortunately, very unequally sensitive to the different colours. The ratios given by the makers are: red, $\frac{1}{16}$; green, $\frac{1}{16}$; blue, $\frac{7}{8}$. And this fact must be borne in mind when interpreting the absence of any record in the blue region. This is not the only defect, however, for these plates also show three dark bands: one between C and D, one between D and E, and one between b and F, the plate being evidently relatively blind in these three places. These three bands are also present in photographs of the solar spectrum, and are therefore not due to the use of acetylene as an illuminant. This, of course, adds greatly to the difficulty of interpreting the absorption bands in these three regions, but has no appreciable effect on the steepness of the curve on each plate shown by each complete series of ten photographs.

The photograph of each day's urine will be seen to consist of twelve records in series. The first eleven of the series were photographed through a regularly diminishing depth of urine from 10 cm. to 0 cm. by steps of 1 cm. Number 11 in each series represents, therefore, the effect produced upon the plate when no urine was interposed, the other conditions remaining constant. In it, in one set of photographs, is shown the D, or sodium line, for purposes of orientation. In the first series of photographs this line is shown in the 12th record. In the second series of photographs the 12th record represents in all the spectrum of burning magnesium ribbon, and is intended to enable the solar line b to be located.

The exposures given to all the records on all the plates were accurately timed with a stop-clock and were equal. The plates were developed with the same developer for equal periods of time. The only variant was the temperature of the dark room, which varied about two degrees Fahrenheit. Thus, the factors likely to cause variation in the results, were kept as small as possible so as

to make the records comparable with each other. There is one other variant, the daily average air temperature and range. I am unable to furnish these data as I am writing this in England, where I have no access to meteorological records. The mean temperatures of the two months, August and September, are, however, very much alike.

Two similar experiments were made upon the same subject: the first from 31st July, 1910, to 7th August, 1910, inclusive, and the second from 10th September, 1910, to 17th September, 1910, inclusive. During both periods, the subject's temperature remained normal, and parasites could not be found in his peripheral blood. Tables showing the results obtained, quantity, colour, reaction, etc., will be found below. In each table the first item represents the control, that is the urine collected before any quinine was administered. I have treated it as unity, or $\frac{10}{10}$, and it is with it that the results obtained for the seven subsequent days must be compared. The ratios thus obtained should then be compared with the photographic ratios.

Now, in estimating the results shown in these tables, the following facts are important:—

1. The amount of absorption required to extinguish the chemical or photographic effect on the pan-chromatic plate in the blue region, is, when compared with that required in the red or green, in the ratio of $\frac{7}{8} : \frac{1}{16}$. A control photograph is, therefore, absolutely necessary.

2. Under normal conditions the percentage of solids excreted daily in the urine is nearly constant, but the quantity of urine passed depending, as it does, on the water constituent, varies greatly.

The amount of solids and pigments in urine is therefore usually in the inverse ratio to the amount of water. It follows, therefore, that if there were in these samples no abnormal increase in the pigments, the ratio borne by the quantity of urine passed each ten hours to the control quantity would be similar to the ratio borne by the photographic records of each day to the photographic control.

For example:—Taking the photographic record of the 10th September, 1910, as control or unity, and comparing the steepness of the photographs of the seven subsequent days with it, a series of ratios can very readily be prepared. Then, taking the quantity of

urine passed on 10th September, 1910, as the control or unity, and comparing the quantities passed on the seven subsequent days with it, another series of ratios can be calculated. Both these series of ratios are shown in the following tables:—

Data Obtained in Experiment I
31—7—10 to 7—8—10

Date	Quantity	Colour	Reaction
31—7—10	640 c.c.	Yellow	Acid
1—8—10	766 c.c.	Pale-yellow	,,
2—8—10	360 c.c.	Pale brown	,,
3—8—10	595 c.c.	Yellow	,,
4—8—10	605 c.c.	,, ...	,,
5—8—10	660 c.c.	,, ...	,,
6—8—10	690 c.c.	,, ...	,,
7—8—10	655 c.c.	,, ...	,,

Ratios

Date	Quantity Ratios	Absorption Ratios	A—Q
31—7—10	$\frac{10}{10}$	$\frac{10}{10}$	0·0
1—8—10	$\frac{12}{10}$	$\frac{10}{10}$	− 0·20
2—8—10	$\frac{5·6}{10}$	$\frac{16}{10}$	+ 1·00
3—8—10	$\frac{9}{10}$	$\frac{14}{10}$	+ 0·50
4—8—10	$\frac{9·4}{10}$	$\frac{14}{10}$	+ 0·46
5—8—10	$\frac{10·3}{10}$	$\frac{15}{10}$	+ 0·47
6—8—10	$\frac{10·7}{10}$	$\frac{11}{10}$	+ 0·03
7—8—10	$\frac{10·2}{10}$	$\frac{10}{10}$	− 0·02

Data Obtained in Experiment II

10—9—10 to 17—9—10

Date	Quantity	Colour	Reaction	Sp. gr.	Temperature	Sp. gr. reduced to 60° F.	Solids in 10 hours
							grms.
10—9—10	595 c.c.	Yellow ...	Acid	1013	84° F.	1021	29
11—9—10	803 c.c.	Pale yellow	,,	1010	80° F.	1018	33·6
12—9—10	235 c.c.	Pale brown	,,	1027	84° F.	1035	19·1
13—9—10	550 c.c.	Amber ...	,,	1021	84° F.	1029	23·6
14—9—10	530 c.c.	Pale amber	,,	1016	84° F.	1024	29·6
15—9—10	550 c.c.	Bright yellow	,,	1014	81° F.	1021	26·9
16—9—10	650 c.c.	Yellow ...	,,	1014	80° F.	1020·6	31·1
17—9—10	541 c.c.	,, ...	,,	1015	80° F.	1021·6	27·2

Ratios

Date	Quantity Ratios	Absorption Ratios	A—Q
10—9—10	$\frac{10}{10}$	$\frac{10}{10}$	0·0
11—9—10	$\frac{13}{10}$	$\frac{6}{10}$	− 0·70
12—9—10	$\frac{4}{10}$	$\frac{18}{10}$	+ 1·40
13—9—10	$\frac{9}{10}$	$\frac{14}{10}$	+ 0·50
14—9—10	$\frac{8·8}{10}$	$\frac{14}{10}$	+ 0·52
15—9—10	$\frac{9}{10}$	$\frac{15}{10}$	+ 0·60
16—9—10	$\frac{10·9}{10}$	$\frac{11}{10}$	+ 0·01
17—9—10	$\frac{9·1}{10}$	$\frac{11}{10}$	+ 0·19

The experience gained while carrying out the previous experiments enabled the last experiment to be performed with greater accuracy in detail, and the data of this experiment are, therefore, somewhat superior to those of the first experiment.

It will be seen, however, that the results obtained in both experiments agree fairly closely in essentials. I believe the following conclusions may be drawn from the results obtained in both :—

1. That a dose of fifteen grains of quinine hydrochloride causes an early increase in the amount of water excreted in the urine.

2. That this increase is followed within twenty-four hours by a marked decrease, which is accompanied by an increase in the excreted pigments.

3. That these pigments consist largely of urobilin (chemical and spectroscopic proofs).

4. That there is an approximate return to the normal elimination of water and pigment, the phase being almost completed in one week.

Subjective Sensations

The subjective sensations following a dose of quinine are best observed by a person who regularly takes one large weekly dose of the drug, for then the sequence of the sensations is observed to be very similar from week to week, and the regularity is sufficiently great to enable the operation of causes other than quinine to be excluded.

1. A dose of fifteen grains of quinine hydrochloride taken on Saturday night is followed, after an hour or so of restlessness, by sound sleep accompanied usually by pleasant dreams. Without quinine sleep is usually dreamless.

2. On the following day, Sunday, there is a feeling of some exhilaration, with deafness, ringing in the ears, some tremor of the hands, and an inclination for bodily and mental activity.

3. On Monday a certain amount of depression of spirits sets in, which lasts through Tuesday. Deafness continues almost unchanged, and sleep is not so sound.

4. The feeling of depression passes away by Wednesday night

or Thursday morning, when the aural effects have considerably subsided.

The increasing use of salts of quinine in malarial prophylaxis has added a continually increasing importance to the estimation of the effects produced by them upon persons who take a daily or weekly dose for long periods of time. The difficulties of the subject are, however, great, for even the condition of the quinine molecule while circulating with the blood is unknown, and there are no convincing proofs of the mechanism by which these salts exert their well-known destructive action on the malarial parasite.

Even the dosage is still a matter for debate, and there are no observations yet available showing the effects exerted by prophylactic doses of quinine upon the health, special senses, or excretions of a healthy person. At present it can only be affirmed that a European living in a malarious country such as West Africa has a choice of two alternatives:—

1. Recurring attacks of malaria.
2. Quinine prophylaxis.

Both alternatives are harmful, and it therefore becomes simply a question of which is least so.

Few experienced persons in West Africa would have any hesitation about deciding for quinine prophylaxis, and there are fewer still who would not wish that some other alternative were available permitting them to choose otherwise, for this long continued daily or weekly use of quinine produces at least in a certain percentage of Europeans some of the following evil effects:—

1. Diminished acuity of hearing.
2. Premature onset of presbyopia.
3. Mental depression.
4. Dyspepsia and skin affections.

So long then as the proximity of an infected native population and of suitable anophelines makes such prophylaxis necessary for European existence, so long the following questions will possess both a practical and theoretical interest:—

I. At what rate is a salt of quinine excreted?

II. Is the amount of excretion in direct or inverse ratio to the amount of the dose?

III. How long does it exert an influence upon the subjective sensations of the person?

IV. What changes does a full dose of a salt of quinine exert upon the urinary pigments, salts, acidity, specific gravity, etc., and does this effect differ in healthy persons and in persons harbouring the malarial parasite?

No. I. The practical importance of this question will be appreciated when the following points are considered:—

1. The whole of a dose of a salt of quinine cannot be recovered from the excretion during the first twenty-four hours after administration.

2. Many persons are taking daily throughout the year a dose of five grains of quinine.

3. A cumulative action of the drug would seem to be a necessary contingency from these premises.

4. Does the drug accumulate in the body, and, if so, how is its cumulative action manifested?

No. II. An answer to this question would enable the safest and most efficient dose to be fixed.

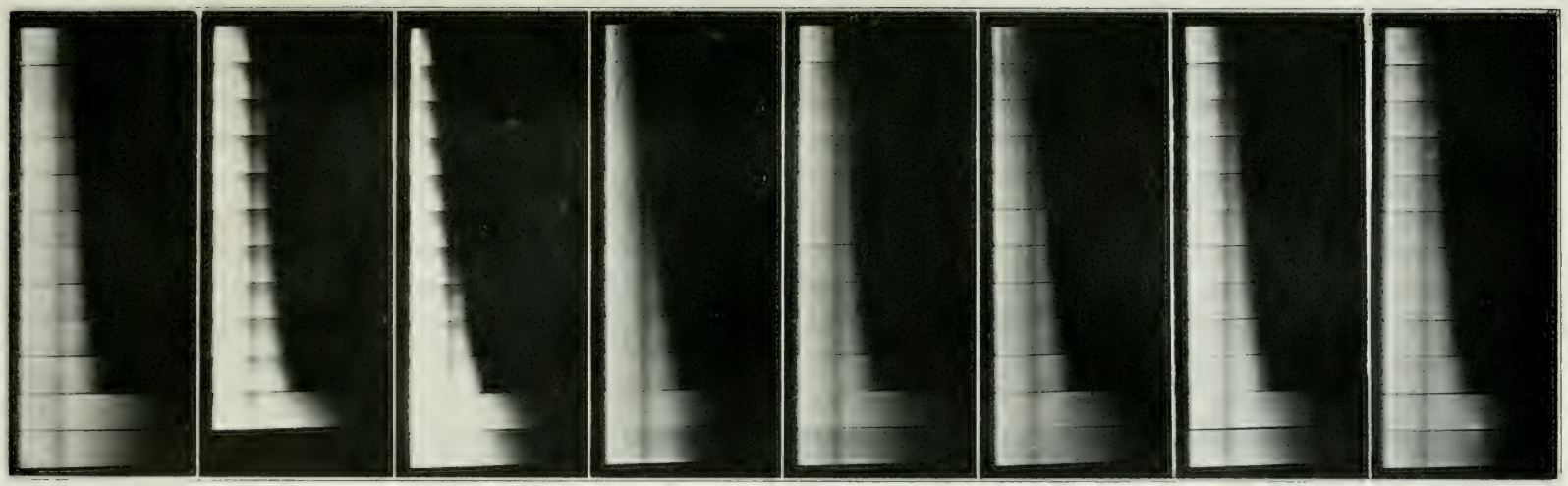
No. III. Answers to this question would enable an estimate to be made of the influence exerted by idiosyncracy, and of its relation to the effect produced upon the special senses.

No. IV. Of all these, the question of the effect exerted upon the elimination of the urinary pigments appears to be most important, especially the effect exerted upon those pigments derived directly from haemoglobin; for an increased excretion in the urine of such pigments would be likely to follow any increased destruction of red blood corpuscles. This applies with special force to the urobilin, the excretion of which, in both urine and faeces, is known to be increased in diseases favouring abnormal destruction of red blood corpuscles. A personal or partial answer to part of question No. IV is furnished by these experiments, but further observation made upon other persons are required to enable the amount of the personal factor or idiosyncracy to be estimated. To exclude the possible influence on the results of malarial infection, similar experiments in a non-malarious country upon persons who have never suffered from malaria are very desirable. It is for this reason that I have explained in the present article so very fully the methods by which my results have been obtained.

FIRST SERIES

31—7—10 1—8—10 2—8—10 3—8—10 4—8—10 5—8—10 6—8—10 7—8—10

10 cm.
9 ..
8 ..
7 ..
6 ..
5 ..
4 ..
3 ..
2 ..
1 ..
0 ..



SECOND SERIES

10—9—10 11—9—10 12—9—10 13—9—10 14—9—10 15—9—10 16—9—10 17—9—10

10 cm.
9 ..
8 ..
7 ..
6 ..
5 ..
4 ..
3 ..
2 ..
1 ..
0 ..
Magnesium

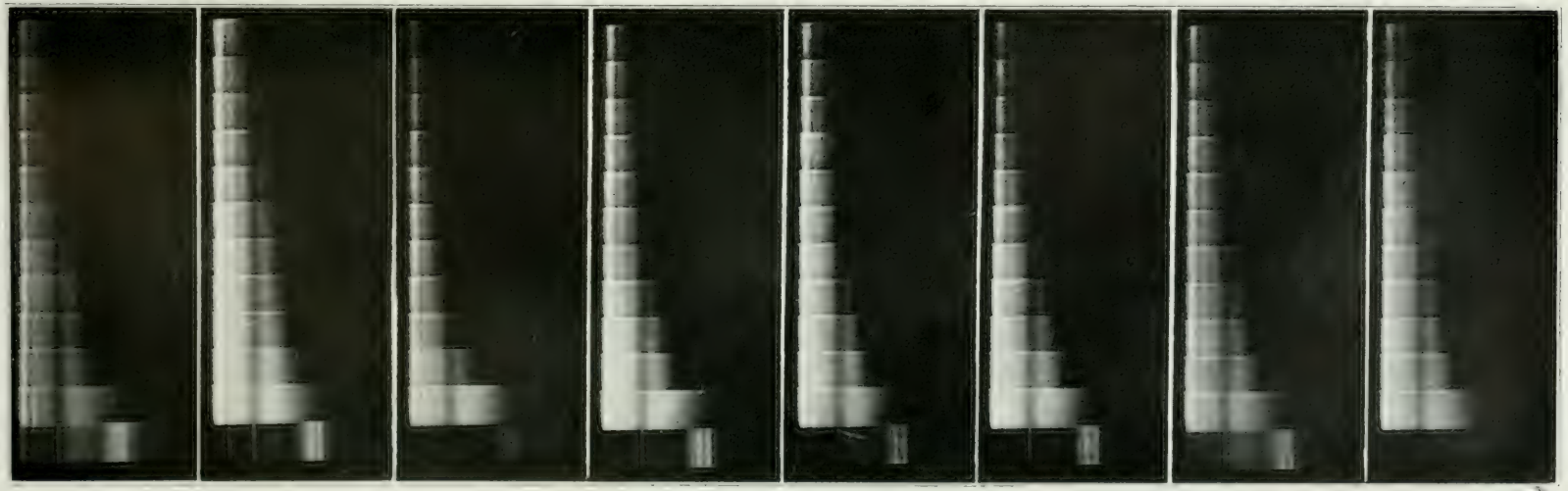


PLATE XVI

THE PASSAGE OF HAEMOGLOBIN THROUGH THE KIDNEYS

BY

WARRINGTON YORKE, M.D.

(*From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine*)

(*Received for publication* 15 *September*, 1911)

If an isotonic solution of haemoglobin obtained from rabbit's red blood cells be injected into a vein of a normal rabbit, the urine is found to be tinged with haemoglobin a few minutes after the injection. Whilst performing experiments devised with a view to studying the mechanism of production of suppression of urine in blackwater fever, the opportunity was afforded of examining a large number of kidneys removed from the animals at various periods after intravenous injection of haemoglobin solutions.

It appeared possible that a careful study of this material might throw some light upon the manner in which haemoglobin passes through the kidneys from the blood stream to the urine.

Attention has already been drawn by Yorke and Nauss (1911) in a previous communication to the fact that under certain conditions suppression of urine follows the intravenous injection of large amounts of homologous haemoglobin. Microscopical examination of the kidneys in such cases shows the condition to be due to occlusion of the renal tubules by casts. These plugs are of different appearance, according as the kidney examined is obtained from animals which have succumbed shortly after the injection of haemoglobin or several days later. When seen in kidneys obtained within a few hours of the injection they are but slightly granular and appear to consist of a fairly homogeneous material, which, when unstained, is of a brownish colour. When stained with various dyes they react in much the same manner as do the red blood cells, e.g., with eosin they stain a brownish pink and with van Gieson a brownish red, whilst with Heidenhain's iron alum haematoxylin method they are dyed a dark blue-black colour.

The casts found in those kidneys where death occurred several days after the intravenous injection of haemoglobin are, as a rule, exceedingly granular. The granules vary considerably in size, from being mere points to granules of about 2 μ in diameter. These granular casts possess in the main the same staining characteristics as the previous variety. Sometimes in the later stages certain of the casts were found to have become in part crystalline.

As to the precise nature of these plugs one is unable to speak with certainty. They are only found after intravenous injection of haemoglobin, and from their appearance both unstained and also after staining with various dyes there appears to be no doubt but that they are derived from haemoglobin. Either they consist of haemoglobin itself supported by a mucoid basis, or they are composed of some closely related derivative. I was able to obtain the iron reaction neither with potassium ferrocyanide and hydrochloric acid (Berlin blue reaction) nor by first treating with ammonium sulphide and then subsequently with hydrochloric acid and potassium ferrocyanide (Turnbull's blue reaction). Possibly the failure to obtain this reaction was because the process of disintegration had not continued sufficiently far for the setting free of iron from albuminous combination to have occurred. It is interesting, however, to note in this connection that the iron reaction was obtained by Werner (1907) in the case of the casts found in the kidneys of several human beings who had succumbed from suppression of urine following blackwater fever. Here, however, the individuals had survived the attacks of haemoglobinuria by over a week. A careful examination of the exact site in which the casts occurred revealed the fact that they were limited to the renal tubules, and were never to be found in the meniscus of Bowman's capsules.

Plugs were frequently found in all portions of the tubule, with the single exception of the glomeruli. The fact that the kidneys removed from over thirty animals, at periods varying from one hour to over eight days after the injection of widely different amounts of haemoglobin, were examined, without discovering casts in the glomeruli in a single instance, is highly suggestive that haemoglobin does not escape through Bowman's capsules.

The possibility suggests itself that haemoglobin might in reality

be excreted by means of Bowman's capsules, but that it is so quickly carried away by the rapid stream of water escaping from the tufts that it is not recognised. However, even assuming Ludwig's theory, that the constituents of the urine are filtered through the capsular tufts in the proportion in which they exist in the blood plasma, to be correct, then in certain of our experiments the glomerular filtrate must have contained at least 8 to 12 per cent. of haemoglobin. If such a solution of haemoglobin be spread in a fairly thick film—considerably less, however, than the thickness of the section of the kidney employed—on a glass slide, fixed immediately before drying has occurred, and then stained with Heidenhain's iron alum haematoxylin or van Gieson, a highly-coloured granular appearance is produced, somewhat resembling the casts seen in the tubules. Such appearances were never observed in the meniscus of the Bowman's capsule in our experimental animals. Moreover, even in those cases in which suppression of urine had occurred and where presumably it would be impossible for any haemoglobin which escaped through Bowman's capsules (sometimes in these cases considerably dilated) to have been washed away, no casts were found in the meniscus of the capsules.

Examination of the kidneys of three blackwater fever patients who had succumbed from suppression of urine revealed an exactly comparable state of affairs. Casts being constantly found in all portions of the renal tubules, except in the meniscus of Bowman's capsules. The same was observed in the kidneys of dogs which died during the passage of haemoglobin resulting from infection with *Piroplasma canis*.

It is difficult to determine the exact portion of the renal tubule which is responsible for the excretion of haemoglobin. Presumably, however, it is the epithelium of the convoluted tubules and possibly also that of the tubes of Henle, as in sections of kidneys removed within a few hours of the intravenous injection of haemoglobin the casts are found to be limited to the cortex, and are not seen in the large collecting tubes of Bellini. Later, however, the plugs are found in the large collecting tubules, but in these cases they have probably simply descended from higher portions of the tubules.

Again, it is interesting in this connection to compare the percentage of haemoglobinuria in relation to the percentage of

haemoglobinaemia observed at definite intervals after intravenous injection of haemoglobin. In Figures 1 and 2 the results of two such observations are given. It is at once apparent from a study of these graphs that the curve representing the percentage of haemoglobin passed in the urine does not run at all parallel with that representing the degree of haemoglobinaemia, for, whereas the latter falls in a comparatively regular manner from the time of injection until the end of the experiment, the former does not reach its maximum until after the lapse of some hours (4 to 5) and, moreover, the curve is irregular, neither attaining its maximum nor falling to zero by regular gradations.

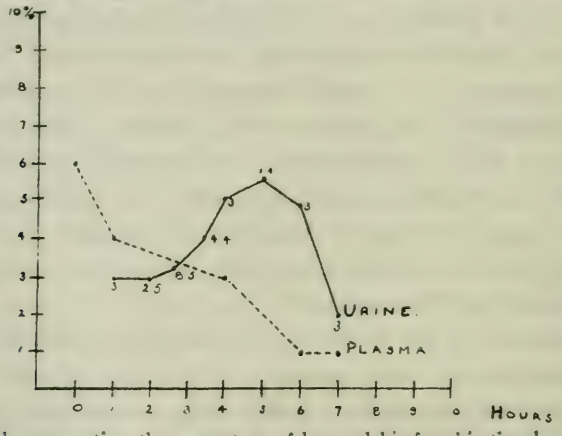


FIG. 1.—Graph representing the percentage of haemoglobin found in the plasma and urine after intravenous injection of an isotonic solution of homologous haemoglobin. The figures along the curve representing the degree of haemoglobinuria indicate the amount of urine passed.

Although the degree of haemoglobinuria varies to a certain extent inversely as the volume of urine passed, nevertheless, this factor alone is insufficient to explain the phenomenon that the maximum amount of haemoglobinuria is not attained for several hours after the intravenous injection of haemoglobin. Assuming that haemoglobin is filtered through the glomeruli, according to Ludwig's view, then one would expect that the percentage found in the urine would depend upon the following two factors. Firstly, it would vary directly with the degree of haemoglobinaemia, and, secondly, it would vary indirectly with the volume of urine passed into the bladder, or, in other words, with the amount of concentration that occurred during the passage of the urine through the convoluted tubules. This, however, does not appear to be the case. Furthermore, it was observed that when several large amounts of

haemoglobin were injected into the same animal at stated intervals, the degree of haemoglobinuria resulting from the last injection was usually much lower than that following the first injection, even though the degree of haemoglobinaemia in this case was not so great as that resulting from the last injection. Moreover, as in these cases the volume of urine excreted after several injections was greatly diminished in amount, owing to partial suppression having occurred, the lower percentage of haemoglobinuria observed could not result from the secretion of a large quantity of watery urine dependent upon decreased absorption of water as it passed through the uriniferous tubules.

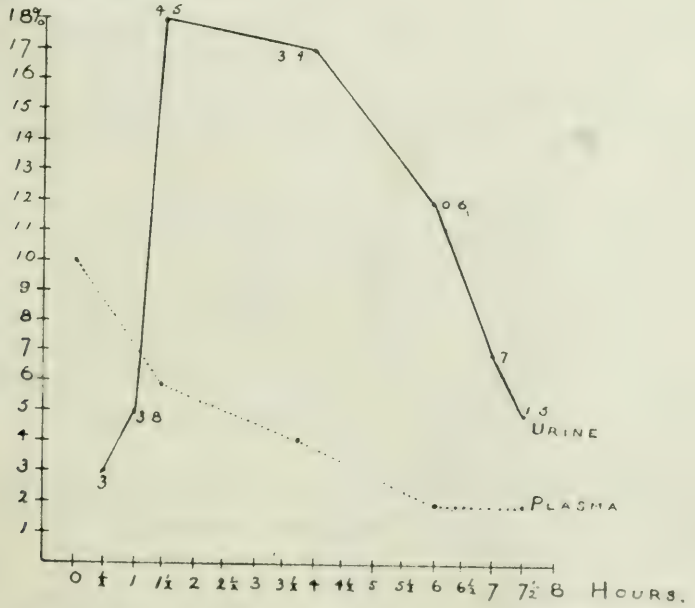


FIG. 2.—Graph representing the percentage of haemoglobin present in the plasma and urine after intravenous injection of an isotonic solution of homologous haemoglobin. The numbers along the curve representing the degree of haemoglobinuria indicate the amount of urine passed.

It would seem that these observations are more in harmony with the view that haemoglobin is secreted by the renal epithelium than that it is filtered through the glomeruli, and, that the amount of haemoglobin eliminated into the urine is dependent upon the activity of the epithelium lining the renal tubules.

There is, moreover, additional evidence which affords support to this view, and that is obtained by examination of the epithelium lining the capsules and various portions of the uriniferous tubules.

On examining sections of the kidneys of pups, which have died from *Piroplasma canis* during the passage of haemoglobin, fixed in formalin and stained with Heidenhain's iron alum haematoxylin method, one is at once struck by the presence of large numbers of darkly staining granules in the epithelium of the renal tubules. These granules vary in size, some being exceedingly small and others much larger, some of the latter being 2 to 3μ in diameter. In sections stained with Delafield's haematoxylin and van Gieson's solution, the granules are of a pale brownish pink colour. These intracellular granules resemble very closely in appearance the granules composing the casts in the lumen of the tubules. They were only found in the cortical region of the kidney, and appeared to be almost entirely limited to the epithelium lining the convoluted tubules. They were never observed in the flattened epithelium of the glomeruli nor in the epithelium lining the collecting tubules of Bellini.

Analogous appearances were observed in the sections of the kidneys of the experimental rabbits, but here the granules were not present in such large numbers as in the dogs.

As in the case of the plugs found in the lumen of the tubules, one is at present unable to decide as to the exact nature of these granules. There appears, however, to be but little doubt that they are derived, in part, at least, from haemoglobin, and are connected with its excretion through the kidney.

Attempts to demonstrate the existence of iron in the epithelial cells lining the tubules of these kidneys were unsuccessful. Here again, however, the explanation may be that the haemoglobin had not sufficiently broken down for liberation of the iron from its proteid combination to have occurred. It is suggestive in this connection to note that Stieda (1893) has described in the kidneys of blackwater fever patients the existence of granules in the epithelium of the convoluted tubes, and states that these granules gave the iron reaction.

REFERENCES

Stieda H. (1893). Einige histologische Befunde bei tropischer Malaria. Centralbl. f. allg. Pathолog. u. path. Anat., IV, pp. 321-331.

Werner (1907). Ueber die Nieren beim Schwarzwasserfieber, Archiv für Schiffs- und Tropenhygiene, XI, Beiheft 6.

Yorke W. and Nauss R. W. (1911). The mechanism of the production of suppression of urine in Blackwater fever. Annals of Trop. Med. and Parasit., V, 2, p. 287.

DESCRIPTION OF PLATE XVII

The tissues were fixed in formalin and the sections stained by Heidenhain's iron alum haematoxylin method.

Fig. 1.—Section of renal cortex of a pup which died from *Piroplasma canis* during the passage of haemoglobinuria. In the epithelium lining the convoluted tubules are numerous darkly stained granules varying considerably in size. The granules are not present in Bowman's capsule.

Fig. 2.—Section of renal cortex of a rabbit four hours after the intravenous injection of haemoglobin. This kidney presents the same appearances as are shown in Fig. 1.

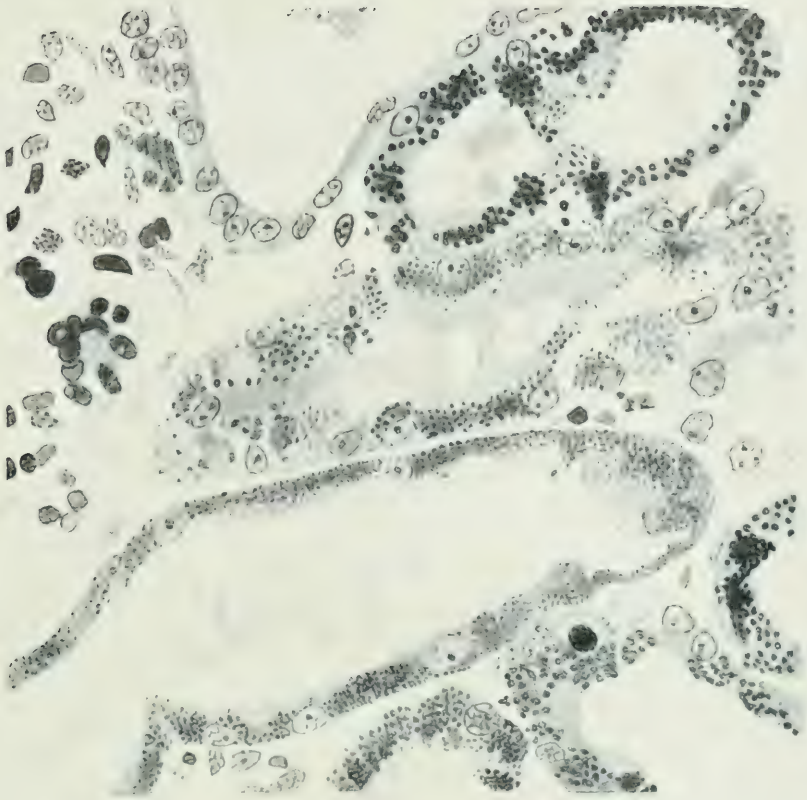


FIG. 1.

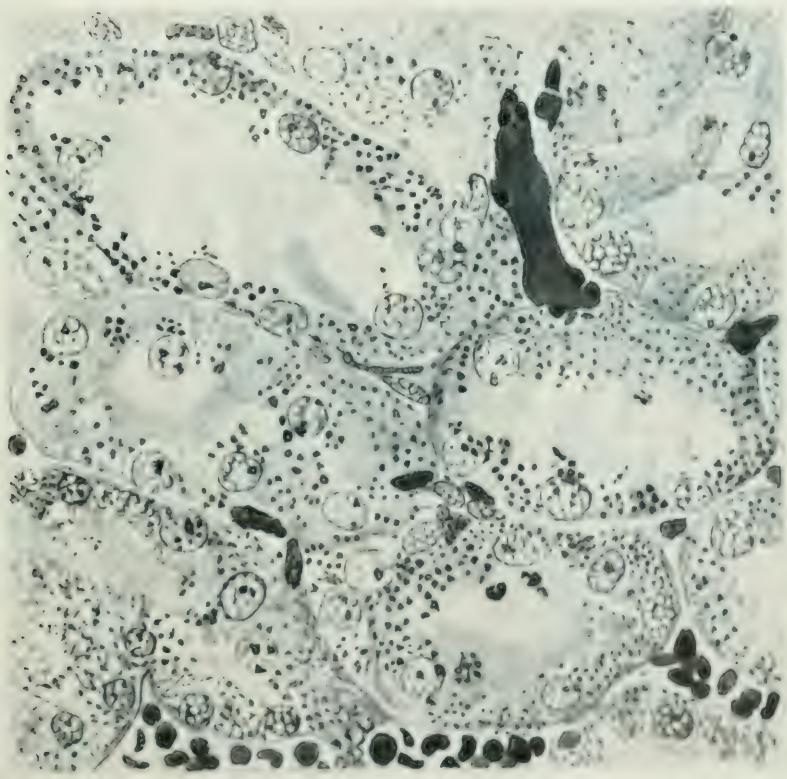


FIG. 2

P. P. Press, Imp.

PSEUDO-RELAPSES IN CASES OF MALARIAL FEVER DURING CONTINUOUS QUININE TREATMENT

BY

SIR RONALD ROSS, K.C.B., F.R.S.,

AND

DAVID THOMSON, M.B., CH.B., D.P.H.

(*Received for publication* 18 *September*, 1911)

There seems to be a current belief that relapses may occasionally occur in cases of malarial fever even during continuous quinine treatment. Caccini (quoted by Ross, 1910) states that a relapse occurred in 15 per cent. of 1,002 cases which had quinine daily. We cannot, however, find accurate data regarding all these relapses, as to whether parasites were found in the blood during the relapse, or how much quinine had been given, or for what period. Again, from various sources it has been stated that cases of malaria occur in the Amazon region which are resistant to quinine treatment; but so far as we know accurate data are not given regarding the presence or absence of parasites during this unsuccessful treatment.

During the past two years we have been studying the effects of quinine on malaria, employing enumerative methods by which we constantly know the number of parasites present in the blood per c.mm. Seventy-five cases studied in this way all showed the remarkable destructive power of quinine towards the asexual malarial parasites. In all cases where quinine was given in doses of ten grains thrice daily, it was almost impossible to find asexual parasites in the blood after three days of the treatment, no matter how numerous the parasites were before the treatment was commenced. In no case did we ever discover a reappearance of these parasites while this dosage was continued.* It would appear to us, that so far, no drug has been found with so great a curative

* Such a case has, however, occurred while this article was in the press. It will be reported later.

power in any disease, as that of quinine in malaria. In our cases of malaria, which came from various parts of the world, including the Amazon, it never failed to show a very remarkable and rapid curative power. It is vastly superior to arsenic, methylene blue, trypan blue, picric acid, etc. We would like, however, to call attention to apparent relapses occurring during quinine treatment. In five (three cases *P. falciparum*, two cases *P. vivax*) or 6·7 per cent. of our cases, a sudden isolated rise of temperature occurred during the quinine treatment, accompanied sometimes with a feeling of cold and slight shivering. No asexual parasites could be detected on prolonged search by thick film during these attacks of fever. On one occasion the blood was examined by one of us and thirteen students for over half an hour, yet no parasites either sexual or asexual could be found. All these apparent relapses were, therefore, non-parasitic relapses. One naturally wonders if these are connected in any way with the malarial infection, or if it is due to the quinine treatment, or if they are mere coincidences, the fever being due to some other cause. We investigated the hospital records of one hundred cases of various diseases, not malarial, and found that similar more or less inexplicable isolated rises of temperature occurred in 17 per cent. of them. The diseases in which these isolated temperature rises occurred most frequently were cases of latent phthisis, cases of valvular heart disease, Bright's disease, chorea, rheumatism and bronchitis, and to a less frequent extent in various other conditions, and in one case of spastic paraplegia. These isolated and more or less inexplicable rises of temperature, therefore, occur in other diseases during treatment as well as in malaria, and it seems possible that they may have no real connection with the original disease. In some cases the temperature could be explained by a sudden and transitory inflammation of the tonsils. One of our cases of malaria had a slight tonsillitis during his pseudo-relapse. Again these rises of temperature in two of our cases did not occur on the proper day, so that they would appear not to be malarial.

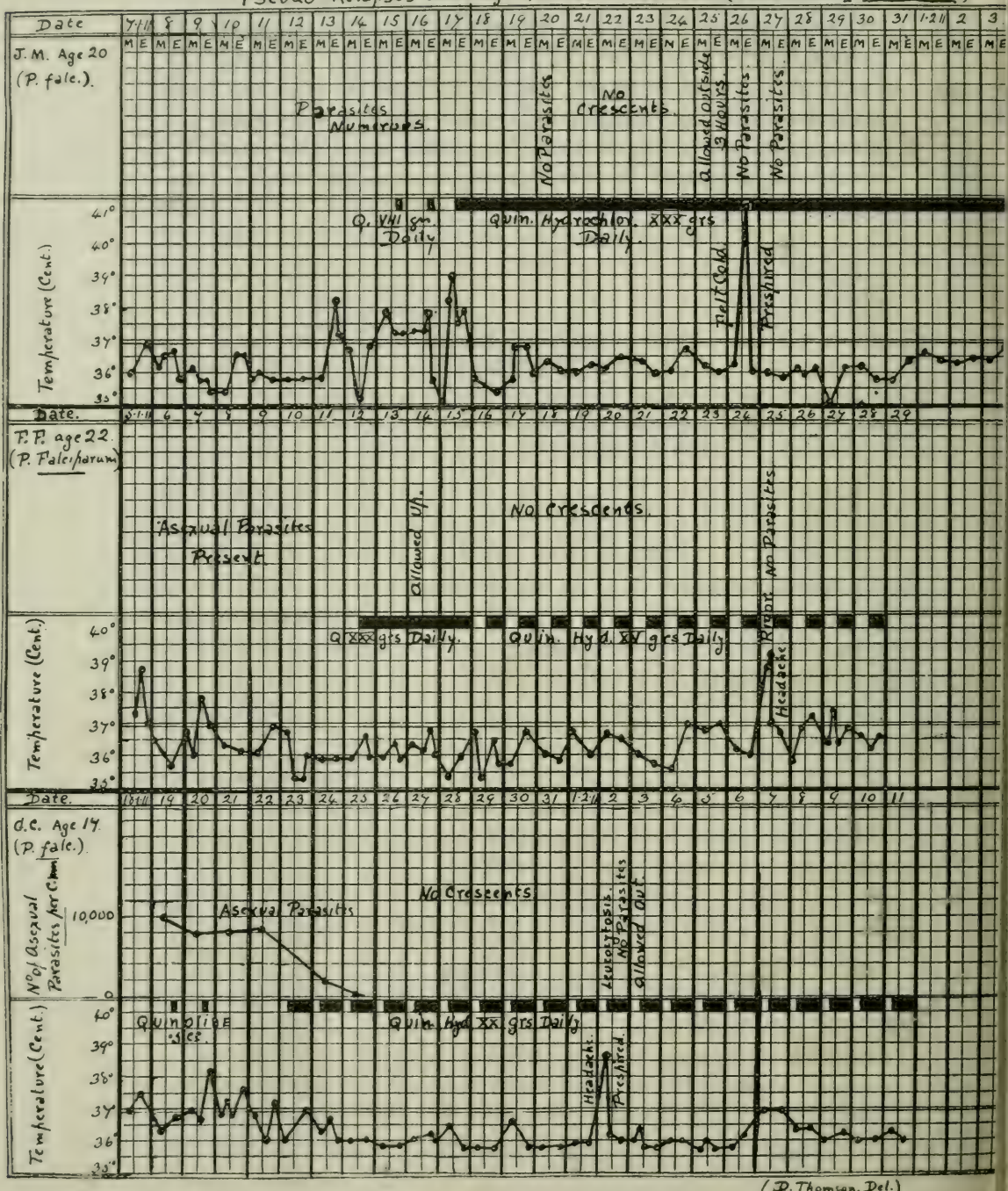
Although numerous non-parasitic rises of temperature with rigors occur in blackwater fever, and although it has been shown by one of us (D. T.) that, from the behaviour of the leucocytes, one might infer that the malarial virus lingers long in the system in spite of

continuous quinine treatment, yet we have no proof that these pseudo-relapses are due to the disease. Some of the relapses during quinine treatment recorded by Caccini may possibly correspond to the 17 per cent. of incidental rises of temperature found in cases not malarial, during treatment in hospital. We therefore think that accurate enumerative observations are needed before the possibility of such relapses, and of cases resistant to quinine, can be fully accepted.

LITERATURE

Caccini (1902). *Journ. of Tropical Medicine*, Nos. 8, 9, 10, 11, 12.
Ross (1910). Prevention of Malaria, p. 138.

Pseudo-Relapses During Quinine Treatment. (Three Cases. P. falciparum.)



(D. Thomson. Del.)

THE TRYPANOSOMES FOUND IN TWO HORSES NATURALLY INFECTED IN THE GAMBIA

BY

WARRINGTON YORKE

AND

B. BLACKLOCK

(*From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine*)

(*Received for publication* 30 *September*, 1911)

CONTENTS

		PAGE
I.	Origin of the Strains	413
II.	Morphology of the Trypanosomes in the Blood of the two Horses	414
III.	Results of Inoculation into Laboratory Animals	417
IV.	Morphology of the Trypanosomes in various Laboratory Animals	417
V.	Comparison of the Trypanosomes	428
VI.	Identity of the Trypanosomes	429
VII.	Conclusion	432

I. ORIGIN OF THE STRAINS

In June, 1911, we were enabled to obtain trypanosomes from two horses, naturally infected in the Gambia. For the first strain we are indebted to Professor Todd, who kindly sent to Runcorn a polo pony, *Horse A*, with a natural infection. Trypanosomes were found in the blood of this animal for the first time in March of this year. For the second strain we are indebted to Sir John MacFadyean, who kindly supplied us with a mouse infected from a horse, *Horse B*, brought to him from the Gambia by Mr. Foster, F.R.C.V.S. By the courtesy of the latter we were permitted to make films from the blood of this horse on its arrival in Liverpool. In this animal the first history of illness dates from about the middle of April, 1911, and trypanosomes were found in its blood for the first time on the 6th of May.

II. MORPHOLOGY OF THE TRYPANOSOMES IN THE BLOOD OF THE TWO HORSES

The parasites found in films made from the blood of the two horses immediately upon their arrival in Liverpool presented remarkable morphological differences. In fact the trypanosome observed in *Horse A* was easily distinguishable morphologically from that infecting *Horse B*. In the former the parasites were of considerable length, uniform, and were furnished with a distinct free flagellum, whereas in the latter they were much shorter and no free flagellated forms were found. In view of this observation we decided to study in detail the parasites infecting each of the animals.

A. *Characters of the trypanosome infecting Horse A.* When examined in fresh preparations the parasite exhibited remarkable activity, dashing across the field of the microscope in a manner strongly suggestive of *T. vivax*. In films fixed in absolute alcohol and stained with Giemsa's solution, the trypanosomes appear to be remarkably uniform in length. The posterior portion of the parasite is broad, and the body gradually tapers towards the anterior end. The nucleus lies near the centre of the animal, but anterior to the broadest portion of the body; it is, as a rule, well defined, but occasionally somewhat diffuse. The blepharoplast, round and distinct, is situated either laterally close to the posterior extremity, or terminally; the membrane is simple and narrow. The parasite has a well-marked flagellum, the free portion varying from 4 μ to 7 μ. As will be seen from Table I, the length* of the trypanosome varies from 17·1 μ to 25·4 μ with an average of 20·6 μ.

In addition to the form just described, occasional short, non-flagellar trypanosomes are encountered. These are, however, exceedingly scarce, only two such being met with in 1,000 trypanosomes counted.

* *Method of Measuring.* In every case the blood films were fixed in absolute alcohol and stained with Giemsa. The parasites were drawn with the aid of the Abbé camera lucida at a magnification of 2,100 diameters, and measured along the middle line of the body from the posterior extremity to the tip of the flagellum. The actual length of the parasite was then determined by dividing by the magnification. The trypanosomes were drawn as they came, only dividing forms being ignored.

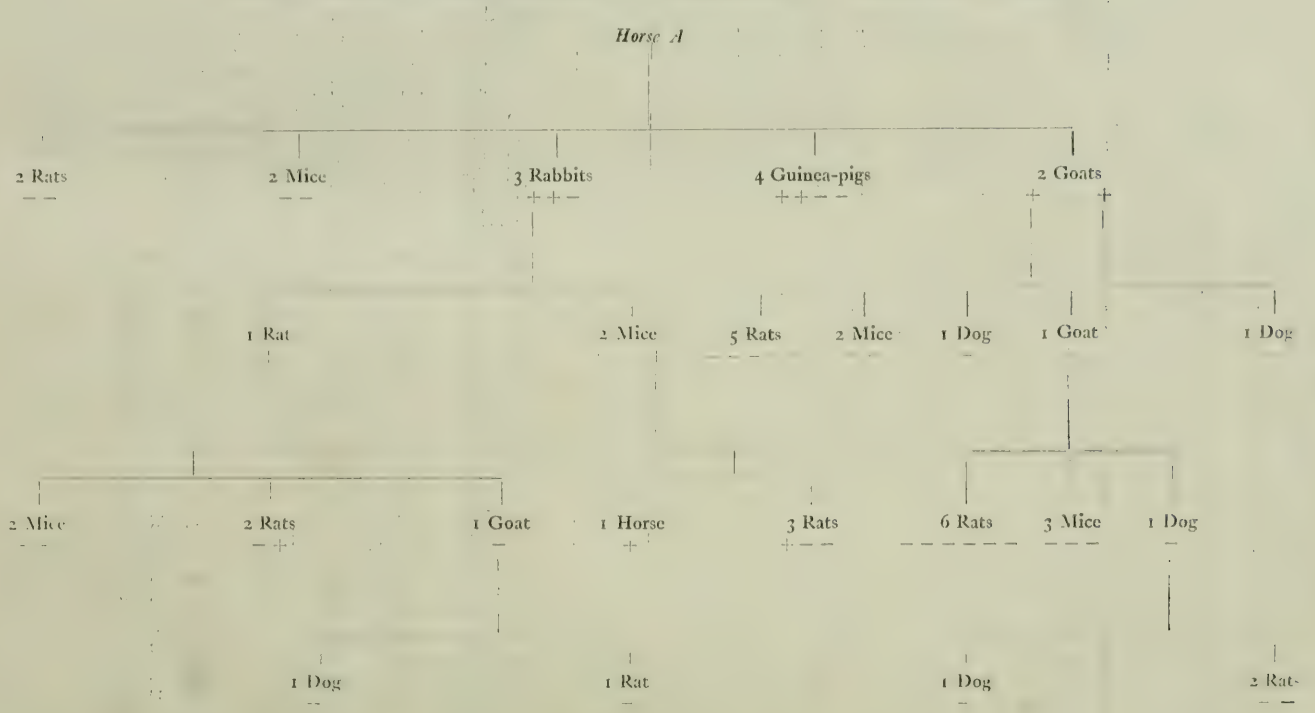
TABLE I.—Measurements of the Trypanosomes in *Horse A*

Date	Animal	No. measured	Maximum	Minimum	Average
June 3	Horse A	20	25·4	18·6	23·2
6	,,	20	23·2	14·0	19·1
9	,,	20	23·2	17·1	20·2
11	,,	20	23·8	19·0	20·0
12	,,	20	23·8	17·1	20·9
13	,,	20	24·2	11·9	21·3
16	,,	20	22·8	18·5	21·0
21	,,	20	23·0	18·0	21·2
22	,,	20	23·8	12·8	20·0
23	,,	20	22·3	18·5	20·1
Total		200	25·4	11·9	20·6

B. *Characters of the trypanosome infecting Horse B.* We had no opportunity of examining this trypanosome in fresh preparations of the blood.

In stained preparations, the parasites found were invariably short and non-flagellated, the undulating membrane being but slightly developed, and the body of the creature prolonged to the extremity of the flagellum. Nucleus oval and central, blepharoplast terminal or latero-terminal. The length of 100 trypanosomes varied from 10 μ to 16·9 μ, with an average of 12·9 μ. No free flagellated forms were met with in the blood of this horse, but it must be remarked that we were only in a position to examine films made on a single day of the infection.

TABLE II. Summary of results of inoculations of the parasite from *Horse A* into Laboratory Animals



− Signifies that the animal did not become infected : + that the animal became infected.

III. RESULTS OF INOCULATION INTO LABORATORY ANIMALS

A. *Animals inoculated from Horse A.* Two rats and two mice inoculated from this horse, at a time when the peripheral blood contained trypanosomes in fair numbers (three to a field, objective DD, ocular No. 4), failed to become infected. Two of four guinea-pigs, and two of three rabbits inoculated with the animal's blood became infected. Two goats showed parasites on the eleventh and fourteenth day respectively, after inoculation. The results of the inoculations made from these animals are given in Table II.

As will be seen from this table, the rats and mice inoculated directly from the horse, and those sub-inoculated with the trypanosome after passage through a goat were all refractory. This applies also to those rats and mice inoculated with the parasites after a second passage through goats. Rats and mice sub-inoculated from a rabbit infected directly from the horse contracted the disease, and from one of these mice a further series of rats and mice was with difficulty infected.

In Table III are given details of our experimental inoculations into laboratory animals.

B. *Animals inoculated from Horse B.* We understand from Sir John MacFadyean that mice inoculated from this horse were easily infected. From a mouse received from him infected with this strain we found that goats, rabbits, guinea-pigs, dogs, rats and mice were easily infected. In mice the incubation period averaged five days and death occurred on the fourteenth day.

IV. MORPHOLOGY OF THE TRYPANOSOMES IN VARIOUS LABORATORY ANIMALS.

Trypanosomes from Horse A. The trypanosome found in goats resembled in every respect that occurring in the blood of the horse. They were all long (17 μ to 26·6 μ), free flagellated forms, with the exception of very occasional short (14 μ to 15 μ) non-flagellated forms. In Rabbit 1467 the parasites averaged from 20·2 μ to 21·7 μ in length, until the day on which death occurred, when only short forms of an average length of 14·7 μ were encountered. In Rabbit 1494, also inoculated directly from the horse, only short forms, with an average length of 11·3 μ to 13·1 μ were found. Similarly only short non-flagellated trypanosomes were met with

TABLE III.—Pathogenicity of the Trypanosome from *Horse A* in Laboratory Animals

No. of Experiment	Animal from which inoculated	Day on which parasites first found in blood	Day on which death occurred	Remarks
Horse A	Unknown	Unknown	June 24th, 1911	
1608 A	Mouse 1535 A	15th	—	Alive on 21st day
GOATS				
1485	Horse A	12th	26th	
1497	,,	10th	44th	
1559	Goat 1497	11th	—	Alive on 62th day
1584	Mouse 1535 B	—	—	Alive on 46th day, parasites never seen
1605	Goat 1559	9th	—	Alive on 30th day
RABBITS				
1467	Horse A	23rd	36th	
1489	,,	—	—	Alive on 110th day ; parasites never seen
1494	,,	19th	—	Alive on 102nd day
1604	Goat 1559	16th	—	Alive on 32nd day
GUINEA-PIGS				
1490	Horse A	—	64th	Parasites never found in blood
1495	,,	19th	22nd	
1519	,,	—	52nd	Parasites never found
1520	,,	18th	—	Alive on 92nd day
DOGS				
1524	Goat 1485	11th	20th	
1553 A	,, 1497	—	—	Alive on 55th day ; parasites never seen
1553 B	,, 1559	—	—	Alive on 48th day ; parasites never seen
1609	,, 1605	—	—	Alive on 20th day ; parasites never seen

TABLE III.—*continued*—Pathogenicity of the Trypanosome from *Horse A* in Laboratory Animals

No. of Experiment	Animal from which inoculated	Day on which parasites first found in blood	Day on which death occurred	Remarks
RATS				
1465	Horse A	—	9th	Parasites never seen
1466	,,	—	6th	,,
1496	,,	—	4th	,, ,,
1526 A	Goat 1497	—	—	Alive on 65th day; parasites never seen
1526 B	,,	—	—	,, ,,
1526 C	,,	—	—	,, ,,
1532 A	,,	—	—	,, ,,
1532 B	,,	—	—	,, ,,
1534	Rabbit 1467	6th	23rd	Few parasites on two days only
1582 A	Goat 1559	—	30th	Parasites never seen
1582 B	,,	—	36th	,,
1582 C	,,	—	—	Alive on 36th day: parasites never seen
1582 D	,,	—	—	,, ,,
1582 E	,,	—	—	,, ,,
1582 F	,,	—	—	,, ,,
1586 A	Mouse 1535 B	25th	—	Alive on 45th day; parasites never seen
1586 B	,,	—	25th	Parasites never seen
1608 B, 1	Mouse 1535 A	8th	—	Alive on 20th day
1608 B 2	,,	—	—	Alive on 20th day; parasites never seen
1608 B, 3	,,	—	14th	Parasites never seen
1610 A	Pup 1553	—	18th	,,

TABLE III.—*continued*—Pathogenicity of the Trypanosome from *Horse A* in Laboratory Animals

No. of Experiment	Animal from which inoculated	Day on which parasites first found in blood	Day on which death occurred	Remarks
MICE				
1498 A	Horse A	—	6th	Parasites never seen
1498 B	,,	—	5th	,,
1527 A	Goat 1497	—	8th	,,
1527 B	,,	—	—	Alive on 90th day; parasites never seen
1535 A	Rabbit 1467	18th	—	Killed for inoculation on 58th day
1535 B	,,	3rd	—	Killed for inoculation on 32nd day
1583 A	Goat 1559	—	7th	
1583 B	,,	—	7th	Parasites never seen
1583 C	,,	—	—	Alive on 47th day
1585 A	Mouse 1535 B	—	30th	Parasites never seen
1585 C	,,	—	24th	,,

TABLE IV.—Pathogenicity of the Parasite from *Horse B* in Laboratory Animals

No. of Experiment	Animal from which inoculated	Day on which parasites first found in blood	Day on which death occurred	Remarks
GOATS				
1587	Mouse 1562 A	13th	—	Alive on 46th day
DOGS				
1624	Goat 1587	7th	—	Alive on 12th day
RABBITS				
1603	Goat 1587	8th	—	Alive on 30th day
GUINEA-PIGS				
1620	Goat 1587	8th	—	Alive on 12th day

TABLE IV.—*continued*—Pathogenicity of the Parasite from *Horse B* in Laboratory Animals

No. of Experiment	Animal from which inoculated	Day on which parasites first found in blood	Day on which death occurred	Remarks
RATS				
1618 A	Goat 1587	9th	—	Alive on 12th day
1618 B	,,	8th	—	,,
MICE				
1554	Mouse (Sir J. Mc.)	5th	10th	
1562 A	Mouse 1554	6th	14th	
1562 B	,,	5th	14th	
1514	Mouse 1562 A	5th	18th	
1619 A	Goat 1587	4th	—	Alive on 11th day
1619 B	,,	5th	—	,,

in the blood of infected guinea-pigs. A pup inoculated from one of the goats showed only short (10·9 μ to 18·7 μ) non-flagellated forms. Only short (9·6 μ to 18 μ) non-flagellated trypanosomes were found in the blood of a rat and two mice sub-inoculated from one of the rabbits.

A detailed account of the measurements of the trypanosomes in various animals experimentally infected is given in Table V.

It will be seen from this table that the parasite varies morphologically in different animals, and sometimes even in the same animal on different days of the infection, in a most remarkable manner.

Trypanosome from Horse B. The trypanosomes found in the blood of mice infected with this strain were short (10·4 μ to 18 μ) non-flagellated, and were similar in appearance to those occurring in the blood of mice infected with *Horse A* strain. Dogs sub-inoculated from the mice contained trypanosomes varying in length from 10 μ to 19 μ, and in rabbits inoculated from the same source the trypanosomes varied from 9·5 μ to 15·2 μ.

In the blood of goats inoculated from the mice the trypanosomes measured from 10·9 μ to 18 μ.

Table VI shows the measurement of the trypanosome in animals infected with this strain.

TABLE V.—Length of the Trypanosome from *Horse A* in Laboratory Animals on various days of the infection

GOATS

No. of Experiment	Animal from which inoculated	Day of disease	No. measured	Measurement in μ			Remarks
				Maximum	Minimum	Average	
1485	Horse A	17th	20	22·8	16·6	20·5	
,,	,,	19th	40	25·4	18·6	22·3	
,,	,,	21st	20	24·7	14·7	20·5	
,,	,,	26th	20	26·0	17·6	22·3	Day of death
		Total ...	100	26·0	14·7	21·6	
1497	Horse A	32nd	50	25·7	14·7	21·7	
,,	,,	34th	20	24·2	19·0	22·3	
,,	,,	37th	60	26·6	17·8	21·3	
,,	,,	46th	50	23·8	19·5	21·5	
		Total ...	180	26·6	14·7	21·6	

TABLE V. *continued*—Length of the Trypanosome from *Horse A* in Laboratory Animals on various days of the infection

RABBITS

No. of Experiment	Animal from which inoculated	Day of disease	No. measured	MEASUREMENT IN μ			Remarks
				Maximum	Minimum	Average	
1467	Horse A	25th	40	25·0	12·7	21·7	Parasites first found in the blood on the 18th day
,,	,,	33rd	20	22·5	18·5	20·2	
,,	,,	34th	60	23·2	17·4	20·9	
,,	,,	36th	50	18·5	10·9	14·7	Day of death
		Total ...	170	25·0	10·9	19·2	
1494	Horse A	38th	20	15·7	10·4	13·1	
,,	,,	40th	20	14·7	9·2	11·3	
		Total ...	40	15·7	9·2	12·2	

TABLE V.—*continued*—Length of the Trypanosome from *Horse A* in Laboratory Animals on various days of the infection

Dogs

No. of Experiment	Animal from which inoculated	Day of disease	No. measured	Measurement in μ			Remarks
				Maximum	Minimum	Average	
1524	Goat 1485	12th	20	17·6	11·1	13·4	
,,	,,	13th	25	16·1	10·0	13·7	
,,	,,	14th	20	17·3	11·1	13·8	
,,	,,	15th	40	18·7	12·3	14·9	
,,	,,	16th	20	18·3	10·4	13·9	
,,	,,	17th	20	15·2	11·4	13·2	
,,	,,	18th	30	17·6	10·9	13·7	
		Total ...	175	18·7	10·4	13·9	

TABLE V.—*continued*—Length of the Trypanosome from *Horse A* in Laboratory Animals on various days of the infection

Rats

No. of Experiment	Animal from which inoculated	Day of disease	No. measured	Measurement in μ			Remarks
				Maximum	Minimum	Average	
1534	Rabbit 1467	14th	20	14·7	9·5	12·3	
,,	,,	14th	30	16·1	9·5	13·1	
		Total ...	50	16·1	9·5	12·8	

Mice

1535 A	Rabbit 1467	19th	20	12·5	9·6	11·8	Parasites first found in the blood on the 18th day
,,	,,	23rd	40	18·0	11·4	13·9	
,,	,,	27th	20	18·0	11·1	13·5	
1535 B	,,	5th	5	15·4	11·9	13·4	
		Total ...	85	18·0	9·6	13·3	

TABLE VI.—Length of the Trypanosome from *Horse B* in Laboratory Animals on various days of the infection

No. of Experiment	Animal from which inoculated	Day of disease	No. measured	Measurement in μ			Remarks
				Maximum	Minimum	Average	
GOATS							
1587	Mouse 1562 A	27th	25	16·6	11·9	14·2	Parasites first found in blood on 13th day
,,	,,	27th	25	16·1	11·9	14·2	
,,	,,	23rd	25	18·0	11·4	14·1	
,,	,,	35th	25	16·6	10·9	14·3	
		Total ...	100	18·0	10·9	14·2	
DOGS							
1624	Goat 1587	8th	50	17·6	10·0	14·1	Parasites first found in blood on 7th day
,,	,,	9th	50	19·0	10·9	13·5	
		Total ...	100	19·0	10·0	13·8	
RABBITS							
1603	Goat 1587	30th	20	15·2	9·5	12·4	

TABLE VI.—*continued*—Length of the Trypanosomes from *Horse B* in Laboratory Animals on various days of the infection

GUINEA-PIGS							
102	Goat 1587	13th	10	13·8	9·5	12·0	
RATS							
1618 B	Goat 1587	18th	50	16·6	10·4	13·0	
,,	,,	19th	50	19·0	9·5	12·9	
		Total ...	100	19·	9·5	12·9	
MICE							
1554	Mouse infected from Horse B	5th	20	15·7	10·4	13·0	Parasites first found in blood on 5th day
,,	,,	5th	20	17·6	11·1	13·7	
,,	,,	8th	20	17·6	10·9	14·3	
,,	,,	8th	20	18·0	11·4	14·2	
		Total ...	80	18·0	10·4	13·8	

V. COMPARISON OF THE TRYPANOSOMES IN THE TWO HORSES

The fact that the trypanosome occurring in *Horse A* is morphologically distinct from that present in films made from the blood of *Horse B* on its arrival in England—both animals being naturally infected in the Gambia—appears to us to be one of considerable interest. The morphological distinction, as we have previously stated, consists in the fact that whereas the trypanosomes infecting *Horse A* were almost invariably free flagellated and uniform in length (average length 20·6 μ), those observed in the blood of *Horse B* were short and without free flagellum.

A second point of interest is that the trypanosomes found in the blood of certain laboratory animals (dog, guinea-pig, rat and mouse), inoculated with the strain derived from *Horse A*, in no way resembled the majority of the parasites present in the blood of this horse. The trypanosome in these animals was short and non-flagellated, and its measurements corresponded closely to those of the parasite present in the blood of *Horse B*. No long free flagellated forms were at any time observed in the blood of these laboratory animals. On the contrary goats infected with the *Horse A* strain exhibited the long free flagellated forms, and only very exceptionally was a stumpy non-flagellar form seen. One of the rabbits infected from this horse was remarkable in that until the last day of the disease the blood contained, with the exception of very occasional short trypanosomes, only long free flagellated parasites. On the last day, however, none of the latter forms was noticed, but a large number of the short non-flagellar trypanosomes was present.

The question now arises, was *Horse A* infected with a single trypanosome capable under certain conditions of assuming a long free flagellum and under other conditions of existing in a non-flagellar state? Either this is so or this animal was infected with two distinct trypanosomes, one of which was a short non-flagellated trypanosome, and the other a long free flagellated trypanosome. The short trypanosome occasionally found in the blood of *Horse A* was similar, morphologically, to the parasite infecting *Horse B*. The point to be determined, therefore, is whether or no

the long forms infecting *Horse A* are of the same species as the short stumpy forms occurring in the same horse and in *Horse B*. We have endeavoured to decide this question experimentally. A series of laboratory animals, goats, dogs, guinea-pigs, rats and mice, inoculated with the blood of a mouse infected with the parasite from *Horse B*, became easily infected, and in all these animals the trypanosome retained its short character, no free flagellated forms being found. Next, the long free flagellated parasite from *Horse A*, as it appeared in the blood of a goat, was inoculated into a series of rats and mice. These sub-inoculated animals did not become infected. Again, the short forms derived from *Horse A*, as seen in the blood of an infected mouse, were inoculated into a horse, goat, rats and mice. Of these animals the goat gave a negative result, parasites never being found in its blood, and test animals—dog, rat, mouse—inoculated with large amounts of its blood, not becoming infected. Some of the mice and rats had a few parasites in the blood for one or two days only; the horse has had parasites for one day. In none of these animals, including the horse, have free flagellated trypanosomes been observed, short forms only, and these in very small numbers, appearing in their blood. Next, certain animals—rabbits, rats, mice—which had proved quite refractory to the parasites, long or short, derived from *Horse A* were inoculated with parasites derived from *Horse B*, and became easily infected. Further experiments are being done.

VI. IDENTITY OF THE TRYPANOSOMES

During the past few years several attempts have been made, for example, by Montgomery and Kinghorn (1908-9) and Laveran (1911), to classify the various pathogenic trypanosomes. Nevertheless, we are partly at a loss to assign a place in any of these classifications to the trypanosomes with which we are at present dealing.

We shall first consider the trypanosome found in the blood of *Horse B*. An examination of it leads one to associate this parasite with quite a definite group. Morphologically this trypanosome is closely allied to *T. dimorphon* in the sense in which the term is

used by Laveran and Mesnil. In its reaction upon laboratory animals also it corresponds definitely with this type of trypanosome. It falls, therefore, into that group which Bruce has designated by the term *T. pecorum*, in which he includes *T. dimorphon* (Laveran and Mesnil), *T. congolense* (Broden), *T. confusum* (Montgomery and Kinghorn), Edington's trypanosome from Zanzibar, the trypanosome from Chai-Chai (Theiler), and Bevan's trypanosome from Southern Rhodesia. We consider, therefore, that the trypanosome in the blood of *Horse B* is probably *T. dimorphon* (Laveran and Mesnil).

We take next the trypanosome infecting *Horse A*. From a study of the parasites in the blood of *Horse A* merely, one would naturally associate the trypanosome with that group of which *T. vivax* is a type, and in which Bruce (1910) has placed the *T. vivax* of Uganda, that from Togoland, the trypanosome from Pordage's ox, and also *T. cazalboui*. The reasons for associating the trypanosome as it appeared in the blood of *Horse A*, with the trypanosomes of this group are its extraordinary activity as seen in fresh preparations, and its morphological appearances in stained blood films, namely its uniformity in size, the peculiar shape of the body, the smallness of the membrane, and the presence of a free flagellum. The average length of this parasite is 20·6 μ, which is less than that given by Bruce for *T. vivax* (23·7 μ), but which corresponds very closely to the length of *T. cazalboui* (21 μ).

The view that this long form of parasite is closely akin to the *T. vivax* group is strengthened by observing the results of inoculations into laboratory animals, because we failed to find this form in the blood of dogs, guinea-pigs, rats and mice inoculated with the blood of *Horse A*, while a trypanosome whose appearance in fresh and stained preparations was identical with that of *Horse A*, was found in goats inoculated from this animal.

Against the view that this trypanosome from *Horse A* is *T. vivax*, we have the fact, mentioned above, that its length is less. In addition to this, however, the fact that we succeeded in infecting a rabbit with this form of trypanosome, militates against such a conclusion. Bruce,* in his paper on the diseases of domestic animals

* Bruce, *loc. cit.*

in Uganda, writes, in reference to *T. vivax*, 'This species of trypanosome is similar to *T. nanum*, in that it is only pathogenic to equines and bovines, and has no effect on the smaller laboratory animals.' Moreover, Laveran* in a recent paper, states that the rabbit, amongst other laboratory animals, is refractory to *T. cazalboui*. We recall here Bruce's statement† that it is probable that *T. vivax* and *T. cazalboui* are the same species.

We pass now to the consideration of the short forms of parasite found in small numbers in the blood of *Horse A*, and in the blood of goats inoculated from it, and in larger numbers in the blood of such dogs, rabbits, guinea-pigs, rats and mice as we succeeded in infecting from the horse or goats. Seen in fresh films of the blood of these animals, and studied in stained preparations, this parasite has considerable resemblance to the *T. pecorum* group.

Against the view that it is identical with *T. pecorum* is the fact that we succeeded in infecting guinea-pigs with the parasite. Bruce‡ found that guinea-pigs were refractory to *T. pecorum*, and Theiler (1909) observed that a trypanosome from Zululand, included by Bruce under the name *T. pecorum*, was also innocuous to guinea-pigs. Whether, however, the apparent insusceptibility on the part of a certain laboratory animal to infection with a trypanosome is sufficient ground for differentiating a parasite from similar trypanosomes known to infect such animal, appears to us to be a very doubtful question. Even the most recent literature contains contradictions on this point, e.g., Laveran§ states that *T. vivax* is pathogenic for the dog and rat. Further, attention has frequently been drawn to the fact that the virulence of a trypanosome can be considerably altered by passage through various animals.

But a glance at Table II will show that the short forms from *Horse A* are, up to now, at any rate, of a very low pathogenicity for several laboratory animals, especially noticeable, perhaps, in the case of mice, rats and dogs, and in this respect they differ entirely, not only from the short forms of parasite found in *Horse B*, but

* Laveran, *loc. cit.*

† Bruce, *T. vivax*, *loc. cit.*

‡ Bruce, *T. pecorum*, *loc. cit.*

§ Laveran, *loc. cit.*

also from our laboratory strains of *T. dimorphon* and *T. pecorum*. Even those animals which became infected, with the exception of a dog, had a chronic infection, and some appear to have recovered.

In regard then to the infection in *Horse A*, we find ourselves confronted by two possibilities—

1. *Horse A* is suffering from a double infection. One trypanosome resembles *T. vivax*, and the other resembles the *T. pecorum* group, but presents remarkable differences from this group in its pathogenicity to laboratory animals.

or

2. *Horse A* is infected with one trypanosome. This trypanosome resembles closely *T. vivax*. But if we are to accept this view, one's conception of *T. vivax* requires to be somewhat modified. In this connection, however, we know that Ziemann (1905) discovered short forms occasionally in *T. vivax* infection.

VII. CONCLUSION

1. We consider the trypanosome found in *Horse B* to be *T. dimorphon*, *sensu* Laveran and Mesnil.

2. The long form in *Horse A* appears to us to be *T. vivax*.

3. As regards the short form found in *Horse A*, we do not feel justified at the present stage in assigning its position. It may be a dimorphon-like trypanosome of low pathogenicity, or simply a modification of the long parasite of *Horse A*.

REFERENCES TO LITERATURE

Montgomery and Kinghorn (1908-9). On the nomenclature of the mammalian trypanosomes observed in North-Western Rhodesia. Annals of Trop. Med. and Parasit., p. 333.

Laveran (1911). Identification et essai de classification des trypanosomes des mammifères. Annales de l'Institut Pasteur, p. 497.

Bruce (1909-10). Trypanosome diseases of domestic animals in Uganda. I, *T. pecorum*. Proc. Roy. Soc., B, LXXXII, p. 468.

Bruce and others (1910). Trypanosome diseases of domestic animals in Uganda. III, *T. vivax* (Ziemann). Proc. Roy. Soc., B, Vol. LXXXIII, p. 15.

Theiler (1909). Sur l'existence de *Trypanosoma dimorphon* ou d'une espèce voisine au Mozambique et au Zoulouland. Bulletin de la Société de Pathologie Exotique, p. 39, Jan. 11.

Ziemann (1905). Nachtrag zum Beitrag zur Trypanosomenfrage, Centralblatt für Bak., p. 662.

DESCRIPTION OF PLATE XVIII

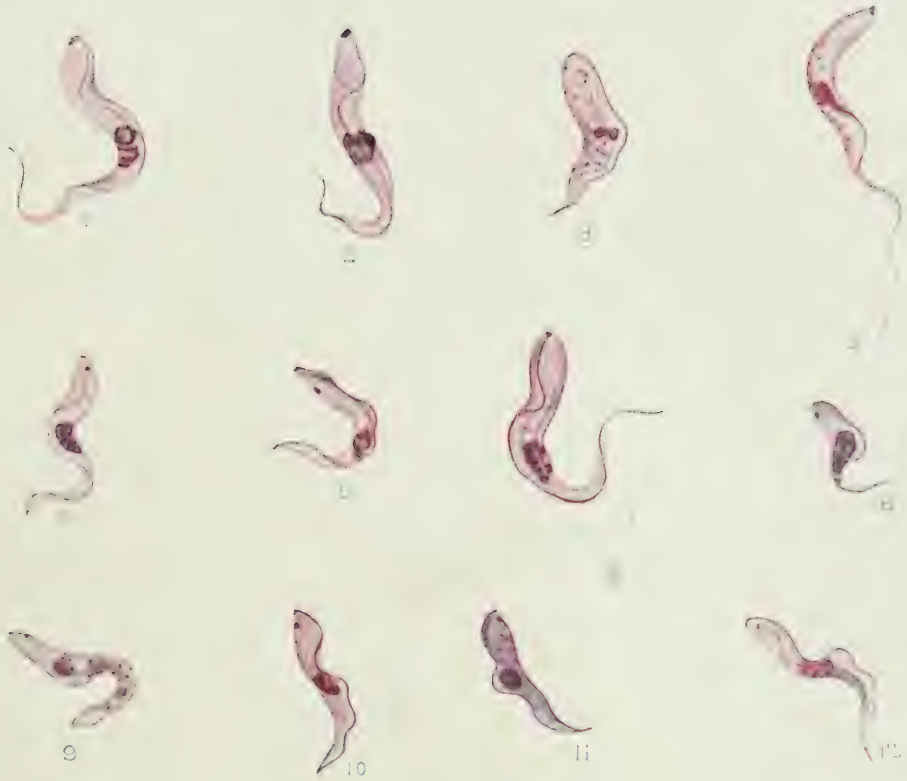
Films stained by Giemsa's method after fixing in absolute alcohol. Figures drawn with Abbé camera lucida, using 2 mm. apochromatic objective and No. 18 compensating ocular (Zeiss). Magnification reduced to 2000 diameters.

Trypanosomes from *Horse A* Strain

Figs. 1-3.—Parasites from *Horse A*.
Fig. 4.—Parasite from Goat.
Figs. 5, 6.—Parasites from Dog.
Figs. 7, 8.—Parasites from Rabbit.
Fig. 9.—Parasite from Guinea-pig.
Fig. 10.—Parasite from Rat.
Figs. 11, 12.—Parasites from Mouse.

Trypanosomes from *Horse B* Strain

Figs. 1-3.—Parasites from *Horse B*.
Fig. 4.—Parasite from Goat.
Fig. 5.—Parasite from Dog.
Fig. 6.—Parasite from Rabbit.
Fig. 7.—Parasite from Guinea-pig.
Figs. 8, 9.—Parasites from Rat.
Fig. 10.—Parasite from Mouse.



Horse A STRAIN



Horse B STRAIN

P. P. Press, Imp.

AN EXAMINATION OF THE CITY OF GEORGETOWN, BRITISH GUIANA, FOR THE BREEDING PLACES OF MOSQUITOS

BY

K. S. WISE, M.B., B.S., B.Sc., D.P.H.

GOVERNMENT BACTERIOLOGIST.

(*Received for publication* 1 *November*, 1911)

The coast line of British Guiana is really an alluvial mud flat, characterised by its flatness and by its lowness of level, which is four to five feet below that of the sea at high water of spring tides. Its soil is heavy alluvial clay, mixed with marine salts and vegetable deposits. The city of Georgetown lies at the angle of this low-lying sea coast and the mouth of the Demerara river. As its level is below that of the sea, the city and the cultivated lands around it require embankments on every side. Sluices on these embankments at suitable times of tide give vent to the surface drainage of the land. The city is intersected by numerous trenches and open drains, varying from two to ten feet wide, which discharge the surface water alone. Water for drinking is rain water collected from the roofs and conserved in large wooden vats and iron cisterns.

There is a further water supply carried by pipes and distributed to the various parts of the city, used mainly for fire purposes, but also for flushing, washing stables, watering, etc. This supply is a brown peaty water, obtained through an open trench from an empoldered area some ten miles from the city. Sewage disposal is mainly by cess-pits; in a few limited areas the pail system is utilised.

In June, 1909, as an outcome of suggestions of Sir R. Boyce, it was proposed to make a close examination of Georgetown, with reference to the breeding places of the myriads of mosquitos always present.

Difficulties arose as to the right of entry of the Staff of the Bacteriological Department on premises, and it became necessary

to await the passing of a Mosquito Ordinance bestowing powers of entry; this was achieved in September, 1910.

This mosquito survey was begun in December, 1910, and was finished in September, 1911, and was carried out by the following persons duly authorised by the Honourable the Surgeon-General:—The Government Bacteriologist, the Assistant Government Bacteriologist, Dr. Duncan, and two skilled Laboratory Attendants specially trained for the work.

In spite of the size and great extent of this city of 60,000 inhabitants, it was decided to enter, examine and report upon all premises, lots, yards, etc., within its boundaries, and no premises were left unvisited The parks and other open spaces, Government buildings, barracks, etc., were visited and reported upon. The trenches in various parts of the city and outskirts were systematically examined as to the conditions under which they were breeding or likely to breed mosquitos. In addition, certain pastures and waste lands lying to the windward side of the city were also surveyed.

Special notice was directed to the kind of mosquito larvae or eggs found, the nature of the places in which breeding was actually occurring, to potential breeding places (i.e., where breeding might occur at times other than that of this visit of inspection).

Records were also made of the condition of the vats, the extent and efficiency of screening and also of the general condition of the yards.

During the progress of this survey, as opportunity occurred, occasion was taken to interest the people in this subject, and numerous (50 to 60) small lectures delivered.

Two thousand five hundred and sixty premises were entered and examined, and of these 1,490 were found to be breeding mosquitos at the time of inspection.

A detailed statement of the above particulars with respect to each lot and half lot occupied has been tabulated and forwarded to the Honourable the Surgeon-General.

The following table shows concisely the various districts of the city, the time of the year when examined, the number of premises, the number and percentage of those breeding mosquitos the number of premises breeding *Stegomyia fasciata*, *Culex fatigans*, *Anopheles* (*Cellia*) *albipes* and other mosquitos:—

District	Date of examination	Premises	Those breeding mosquitos	Percentage	Stegomyia fasciata	Culex fatigans	Anopheles	Other Mosquitos
Kingston	Dec., 1910-Feb., 1911	131	71	54·2	58	27	0	0
Stabroek	Feb., 1911 ...	126	64	50·7	58	9	0	*Culicelsa taeniorhynchus* 7
Queenstown	Feb., 1911 ...	101 much unoccupied land	78	77·2	70	15	2 premises frequent in unoccupied land	*Culicelsa taeniorhynchus* 9
Charlestown	Mch.-May, 1911	254	198	77·9	181	20	1	*Culex confirmatus* 1 *Culicelsa taeniorhynchus* 2
Alberttown	May-July, 1911	269	221	82·1	202	38	8	*Aedomyia squamipenna* 2
Cummingsburg	Aug.-Sept., 1911	245	152	62·0	138	35	2	*Culicelsa taeniorhynchus* 1
Bourda	Sept., 1911 ...	399	131	32·9	125	15	0	0
Lacytown	Sept., 1911 ...	319	133	41·6	125	12	0	0
Robbstown and Newtown	Sept., 1911 ...	48	12	25·0	11	0	1	0
Werken Rust	Sept., 1911 ...	333	201	60·3	175	37	3	
Wharves, Riverside ...	Sept., 1911 ...	53	18	33·8	18	1	0	0
Wortmanville	Sept., 1911 ...	182	145	79·6	138	34	2	*Melanoconion atratus* 1
Railway line	Sept., 1911 ...	100	66	66·0	63	7	2	*Aedomyia squamipenna* 2
Total	—	2560	1490	58·3	1362	250	21	25

From this it will be seen that all parts of the city are affected alike; premises of the rich and poor are equally involved.

The following table indicates the number of vats screened, defectively screened and totally unscreened, the number of premises with potential breeding places, and also the number of these premises kept in a disgraceful condition:—

Ward	Vats screened well	Vats screened defectively	Vats unscreened	Potential breeding places	Disgraceful yards
Kingston	83	51	6	73	13
Stabroek	103	102	8	70	6
Queenstown	64	70	8	90	36
Charlestown	184	155	12	204	26
Alberttown	197	122	0	241	8
Cummingsburg	646	284	13	214	18
Bourda	333	138	13	216	24
Lacytown	283	213	12	185	22
Robbstown and Newtown	43	15	2	14	8
Werken Rust	421	170	6	275	68
Wharves, Riverside ...	99	20	6	28	4
Wortmanville	145	51	1	162	34
Railway line	4	5	1	76	4
Totals	2605	1396	88	1848	271

The Anopheline mosquitos (nearly always *Cellia albipes*, occasionally *Cellia argyrotarsis*) were found breeding in nearly all trenches, *where overgrown with vegetation*, both in the city and environs. Where no vegetation was permitted no Anopheline larvae were discovered. These mosquitos were also found breeding in the hollowed-out stumps of trees which had been cut down in the lots and by the roadside. Numerous Anopheline larvae may be found during the rainy seasons, in the small grass-grown cross-drains of the Queenstown district. They may occasionally be met with in cocoanut shells and in the grass-grown pools and trenches around the barracks.

The developmental forms of the various Culicine mosquitos were most frequently met with, and the breeding places may be summed up as follows:—

Stegomyia fasciata (syn. *S. calopus*) was found breeding in vats, water barrels, tubs, tins, pots, cisterns and defective gutters in the majority of the premises infected, including the river wharves and the premises on the railway line. The breeding places were always close to human habitations, and were never found in trenches or unoccupied land.

Culex fatigans was found breeding in vats, tanks, barrels, tubs and old tins on about one-tenth of the premises visited.

Culex confirmatus was found once in a pond in Charlestown.

Melanoconion atratus was found breeding in open trenches and ponds covered with vegetation such as in Thomas Street, in the pools of the railway line, the 'Governor's fish pond,' and the trenches by Kelly's dam.

Culicelsa taeniorhynchus was a frequent habitant of trenches, more especially those by the military barracks, Kelly's dam, and the sea wall.

Of the Aedine mosquitos only one specimen was found, viz.: *Aediomyia squamipenna*. This was found breeding in trenches and ponds covered by vegetation in Thomas Street, the 'Governor's fish pond,' and by Kelly's dam.

In the vats, barrels and tubs, the larvae found were almost always *Stegomyia fasciata* or *Culex fatigans*.

In old tins, broken crockery, bottles, calabashes, cocoanut shells, fallen palm sheaths, etc., almost always the larvae found were those of *Stegomyia fasciata* and *Culex fatigans*. Occasionally, under these circumstances, *Culex similis*, *Culicelsa taeniorhynchus* or *Culex confirmatus* were found.

In the trenches, when cleansed of vegetation, no evidence of mosquito breeding was found, but *where vegetation covered the surface* numerous larvae of Anophelines and *Aediomyia squamipenna* and *Melanoconion atratus* were discovered.

The 'Governor's fish pond' illustrates excellently the influence of vegetation in giving protection to the larvae from the ubiquitous predaceous fish (*Girardinus poeciloides*). When cleared of vegetation, frequent examination failed to reveal larvae. The presence of larvae was coincident with the growth of vegetation.

In the trenches and open lands to the windward side of the city, where vegetation is associated with the water, larvae of Anophelines, *Culicelsa taeniorhynchus*, *Aediomyia squamipenna* and *Melanoconion atratus* were readily found.

Those premises on which potential breeding places were found numbered 1,848, or 70·9 per cent. of all premises visited. While many such potential breeding places were defectively screened vats, old pots and tins, the great majority were barrels. On no less than 1,203 premises were one or more barrels holding either rain water or the peaty water from the Lamaha Conservancy. On only 11·5 per cent. of these premises was any attempt at screening a barrel shown, and on the greater number of this small minority the screening (generally very defective) was only vouchsafed to one or two out of numerous barrels.

The systematic inspection of the vats (this does not refer to iron cisterns or tanks) shows that 63·9 per cent. were effectively screened. One thousand three hundred and ninety-six vats had more or less defective covers nullifying any beneficial effect of screening. In several instances pieces of wood were used to prop open an otherwise efficient vat cover, thus rendering the purpose of the cover useless.

In many vats an efficient cover had been put on and then completely neglected; the sun drying the unseasoned wood caused warping and the production of large cracks.

Eighty-eight vats were found in which there was no attempt at screening.

A most serious disadvantage under which the inhabitants of this city allow themselves to labour is the excess of vegetation and litter in the yards and lots. No less than 261 premises were kept in a condition which can only be described as disgraceful. The exclusion of the sun keeps the premises damp and dark, and provides excellent cover and breeding places for mosquitos, rats, and other noxious insects, vermin, etc. The exclusion of fresh air and the general dampness encourage and aid the spread of tuberculosis.

The extraordinary collection on some premises of old tins and other worthless rubbish giving rise to stagnant water has to be seen to be believed.

The city of Georgetown is richly supplied, not only with mosquitos but also with convenient and comfortable breeding places. It seems almost impossible to realise that during the wet season over 70 per cent. of all premises in this city are breeding countless myriads of these pests, and that during the dry season nearly 60 per cent. are equally incriminated.

This state of affairs, occurring as it does in a city priding itself on being up to date, is scarcely to be credited, and reveals the urgent necessity for vigorous and prompt action by those responsible for the health of the city.

Undoubtedly the presence of numerous unscreened barrels contributes most to this state of affairs, and the close screening of a barrel and the provision of a tap near the bottom should be a *sine qua non* for even tolerating their existence.

A CASE OF HUMAN TRYPANOSOMIASIS IN NYASALAND WITH A NOTE ON THE PATHOGENIC AGENT

BY
HUGH S. STANNUS
AND
WARRINGTON YORKE

(*Received for publication* 9 *November*, 1911)

In October, 1908, the first case of human trypanosomiasis was discovered in Nyasaland. A native who was examined in consequence of his having accompanied his employer through the Congo was found to be infected. He presented no symptoms, and from that time to the present, during which period treatment has been given, he has preserved excellent health. During the next year further cases came under observation which, in the absence of any history of infection in the then known 'palpalis areas' gave rise to suspicions as to the local conditions in Nyasaland. Investigations carried out to determine the presence or otherwise of *Glossina palpalis* in the Protectorate proved negative, and uncertainty persisted.

In the second half of the year 1910, however, the occurrence of a case in a European, whose movements were exactly known, followed by the discovery of some forty cases in a localised area in the country, has brought into relief the subject of human trypanosomiasis in Nyasaland.

For reasons which the above remarks make obvious, the case of this European is now published in some detail, and the question of the identity of the pathogenic agent dealt with in view of the fact that a trypanosome derived from a case of sleeping sickness, contracted in North-Eastern Rhodesia, has been shown by Stephens and Fantham (1910) to present certain morphological peculiarities, and also by Yorke (1910), a pathogenicity in experimental animals, which differentiate it from all known strains of *Trypanosoma gambiense*.

The history of the patient is as follows.

P. R., aged 60, male, of Beaufort West, Cape Colony, came to Nyasaland on a shooting trip; he landed at Chinde, and travelling by the Zambezi and Shiré rivers, passed up through Blantyre on July 27th, 1910, on his way to Angoniland, arriving at Manda on July 1st, where he remained eleven days. On July 1st he travelled to Mpastso, via Mpunzi and Dedza, thence to Mt. Dzobwe, forty miles to the westward, and back again; leaving again on the 19th he went to Mpinzi, remaining in the neighbourhood of the Diampwe river until the 23rd, but reaching Nkoma the following day. Here he remained till the 28th, when he proceeded to Mvera, where he again spent some days. Here, on August 13th, he saw a case of sleeping sickness, a native whom he photographed as he lay in the open, but did not approach within a few yards of him. He left Mvera for the lower country towards the shores of Lake Nyasa for the shooting. On the 14th he was at Maganga's village; the 15th and 16th were spent in the Patsanjoka dambo, a large area of partly uninhabited country, but containing much game. On the 17th he was at Nsarula, a village on the Lintipi river. During these three days all the party were bitten by tsetse flies (species unrecognised), but the patient suffered most severely owing to an exposed neck. A return was made to Mvera on the 18th, and the next day he went on to Kongwe, where he complained that the bites on the neck were painful, he, however, continued his journey the following day (20th), and reached Nambamba on the 22nd. During the journey he had not appeared very well, and on the day of arrival at Nambamba gave up shooting owing to the pains in the neck. The following day his neck was examined by one of his companions, who found a 'lump about the size of a shilling, rather light in colour surrounded by a dark purple ring,' in the sub-occipital region where pain had been complained of; at the centre of the lump was what appeared to be a puncture due to the bite of a fly. On the 24th, at Sante, on the Bua river, pain in the neck was shooting up into the head, causing severe headache. The lesion on the neck was 'hard as if an abscess were developing,' so poultices were applied; the temperature was found to be 102·5° F. The following day the swelling of the neck had extended; the face was puffy, speech impeded and the temperature remained at 103° F. On the

28th the lump had disappeared, but the oedema persisted. The temperature varied between 102° and 104° F. during the next few days, and the condition remained unchanged. On the last day of August the blood was examined, and trypanosomes found in large numbers. An injection of six grains of atoxyl was immediately given (11 a.m.), followed twelve hours later by a critical rise of temperature to 108° F., and then a fall to 99° F. the following morning, when atoxyl (six grains) was again given.

Progress of case.

Rapid improvement took place to commence with, and by the end of a few weeks patient was able to sit up all day and walk a little; the appetite was good, and there was little in the way of symptoms; constipation, an old trouble, was marked.

On September 24th there was an attack of adenitis, involving the posterior cervical glands of both sides, which responded to treatment by the application of Linimentum A.B.C.; one large gland, about the size of a filbert-nut, persisted for one week. There were several recurrences of this adenitis during the next two months.

A characteristic rash made its appearance on several occasions, the first was on the 78th day, lasting two or three days. The temperature showed a marked periodicity during the earlier part of the illness; it rose on Tuesday of each week and remained between 102° and 104° F. till Thursday, followed by a period of normal temperature till the following Monday; later the maxima showed a steady fall and the apyrexial periods became progressively longer, two days low fever and five days free in each week. The accompanying temperature chart shows these points.

Preceding or accompanying the rises of temperature there were constitutional symptoms, the patient feeling ill and bilious, often with vomiting. Anaemia and emaciation, general cachexia, were progressive though the mental condition remained good. With the constitutional symptoms patient retired to bed, but at other times sat up in a chair.

Such is a short account of the case up till the time he left the country on January 13th, 1911, for South Africa, where he died in April of the same year.

One fact is worthy of special note, and that is the time which elapsed between the patient being bitten by tsetse fly and the onset

of symptoms which practically fixes the incubation period at between six and ten days.

The treatment given was as follows:—For the first five weeks six grains of atoxyl were injected intra-muscularly on Thursday and Friday of each week. For a second period of five weeks three grains of atoxyl were given every 3rd and 4th day. From the 11th to the 18th week soamin (ten grains) was administered on two successive days in every other week, and perchloride of mercury once in the intervening weeks. As the injections of mercury were very painful, and were not followed by any improvement, they were discontinued and soamin alone administered in ten grain doses every Thursday and Friday.

Blood. On the first occasion when the blood was examined trypanosomes 'swarmed,' the field resembling the blood of a rat heavily infected with *T. gambiense*. Their numbers diminished with treatment, and on some occasions none were found in the course of a fairly quick examination. Though it was impossible to make careful enumerations of trypanosomes, still it was noticed there was a certain periodicity in their numbers corresponding to the temperature curve, fewness being associated with the apyrexial intervals, larger numbers with the rises in temperature.

Morphological features of the parasite in the blood of the patient. Unfortunately, the material at our disposal was rather limited, consisting of a slide of the blood made on August 31st, the day on which the disease was first diagnosed, a couple of slides made on November 21st, and one on January 4th, when the patient passed through Zomba on his way to Chinde. The slide made on August 31st contained numerous trypanosomes, and was sent to the Sleeping Sickness Bureau and examined by Sir David Bruce, who found that the parasite did not differ in any way from the Uganda *T. gambiense* (1910).

In the specimens prepared on November 20th and January 4th trypanosomes were more scanty, and the parasites could not be distinguished in any way from *T. gambiense*. The parasite presented the characteristic dimorphism, slender forms with long free flagella, short stumpy forms with no free flagella, and intermediate forms being found.

The Morphology of the parasite in animals experimentally

infected. The parasite was also studied in the blood of several experimental animals, and the results, in short, communicated by us (1911) to the Royal Society.

An English rabbit, bred in Nyasaland, previously shown to be free from any trypanosomal infection was infected with the trypanosome by subcutaneous inoculation with a small quantity of the patient's blood, taken on the 135th day, and subsequently sub-inoculations were made into a monkey (*Cercopithecus sp.*) and a goat. The animals were kept in fly-proof cages. The rabbit and monkey both became heavily infected and exhibited numerous parasites in the peripheral blood, whereas in the goat trypanosomes were only occasionally found in small numbers.

Examination of the parasite in the blood of the rabbit and monkey at once revealed the same morphological peculiarity which was observed by Stephens and Fantham in the trypanosome obtained from a case of sleeping sickness contracted in the Luangwa valley of North-East Rhodesia, i.e., among the stout and stumpy forms some had the nucleus at the posterior (non-flagellar) end (Plate XIX, figs. 5-12 and 14-16). When the parasites were numerous it was found that these posterior nuclear varieties formed from 1 to 4 per cent. of the total number of trypanosomes present. Posterior nuclear forms were only observed when the blood contained fairly numerous parasites. They measured 17 to 22 μ long.

The other parasites found were indistinguishable from *T. gambiense* and exhibited the usual dimorphism. The cytoplasm of many of the parasites observed in the blood of the monkey was vacuolated in a remarkable manner, sometimes as many as five or six large clear vacuoles were seen in a single parasite (figs. 3, 4, 5 and 9). In many of the parasites the cytoplasm contained large coarse granules. The posterior extremity of many of the parasites, especially those in which the nucleus was situated posteriorly, presented a blunt 'cut away' appearance. Parasites similar to those described by Stephens and Fantham as 'snout' forms were likewise observed, but they did not appear to be a prominent feature. After finding these posterior nuclear forms in the blood of the rabbit and monkey, we re-examined carefully the slide of the blood of the patient himself, made on August 31st, at a time when the parasites were numerous. A prolonged search failed to reveal the presence of

any typical posterior nuclear forms, but several dividing forms were seen, in one of which the nuclei were situated close to the blepharoplast (figs. 16 and 17).

Pathogenicity. Unfortunately, absence of laboratory animals prevented the investigation of this point. The three animals (rabbit, monkey and a goat) inoculated with the strain, by one of us (H. S. S.) in Nyasaland, were all easily infected.

In the case of the *Rabbit* the temperature rose before the completion of the sixth day, and trypanosomes were found for the first time in the peripheral blood on that day. The animal was listless and showed a disinclination to feed. The temperature reached a maximum of 105° F. on the 10th day and then fell, reaching 100° F. on the 12th day, rising again immediately afterwards. There was a second fall on the 23rd day and again a rise till the death of the animal on the 27th day. Loss of appetite, rapid emaciation and anaemia were noted, with onset of paralysis during the twenty-four hours preceding death, the muzzle resting on the ground, then involvement of fore-quarters and stertorous breathing; other symptoms noticed, usually seen in rabbits suffering from trypanosomiasis, were oedema of face and purulent discharge from the nose and ears. During the last six days trypanosomes were present in considerable numbers.

Monkey. Inoculated from the rabbit. Parasites appeared in the peripheral blood on the 7th day. During the next week trypanosomes were present in considerable numbers, but later they were scanty though persisting till the time of its death, on the 58th day. There was an irregular pyrexia, the temperature varying between 99° and 102° F. Emaciation and anaemia were progressive though appetite was well maintained, loss of hair about the eyebrows was noticed, and though towards the end it became very quiet, it was not somnolent, death was preceded by eight hours of coma.

There was distinct auto-agglutination of the red blood cells.

Goat. Inoculated from the rabbit also, developed the disease. Trypanosomes found in the blood on the 15th day and on subsequent occasions, but always in very small numbers until the death of the animal on the 28th day.

Symptoms were intermittent pyrexia, extreme wasting, anaemia, oedema of the face and, towards the end, paresis of forequarters.

The rapidity of the course which the disease took in this animal is worthy of remark, and is in accordance with the observations of one of us (W. Y.) working with the parasite obtained from a case of trypanosomiasis infected in the Luangwa valley.

Conclusions. As a result of our observations we are of opinion that the trypanosome in question is not *T. gambiense*. On the other hand this trypanosome resembles very closely *T. rhodesiense*, and is probably identical with it.

The disease was contracted in a district (Dowa sub-district of Central Angoniland) where *Glossina palpalis* has never been found, but where *Glossina morsitans* is known to exist in large numbers. It appears probable, therefore, that this trypanosome (*T. rhodesiense*) is a distinct species which is capable of transmission by some other agent than *Glossina palpalis*, probably *Glossina morsitans*.

LITERATURE

Stephens and Fantham (1910). 'On the Peculiar Morphology of a Trypanosome from a Case of Sleeping Sickness and the Possibility of its being a new species (*T. rhodesiense*).' Proc. Roy. Soc., B, Vol. LXXXIII, p. 28, and Ann. Trop. Med. and Parasit., IV, pp. 43-352.

Sleeping Sickness Bulletin' (1910), Vol. II, No. 21, p. 346.

Stannus, H. S., and Yorke, W. (1911). 'The Pathogenic Agent in a case of Human Trypanosomiasis in Nyasaland.' Proc. Roy. Soc., B, Vol. LXXXIV, pp. 156-160.

Yorke, W. (1910). 'On the Pathogenicity of a Trypanosome from a case of Sleeping Sickness contracted in Rhodesia.' Ann. Trop. Med. and Parasit., Vol. IV, pp. 351-368.

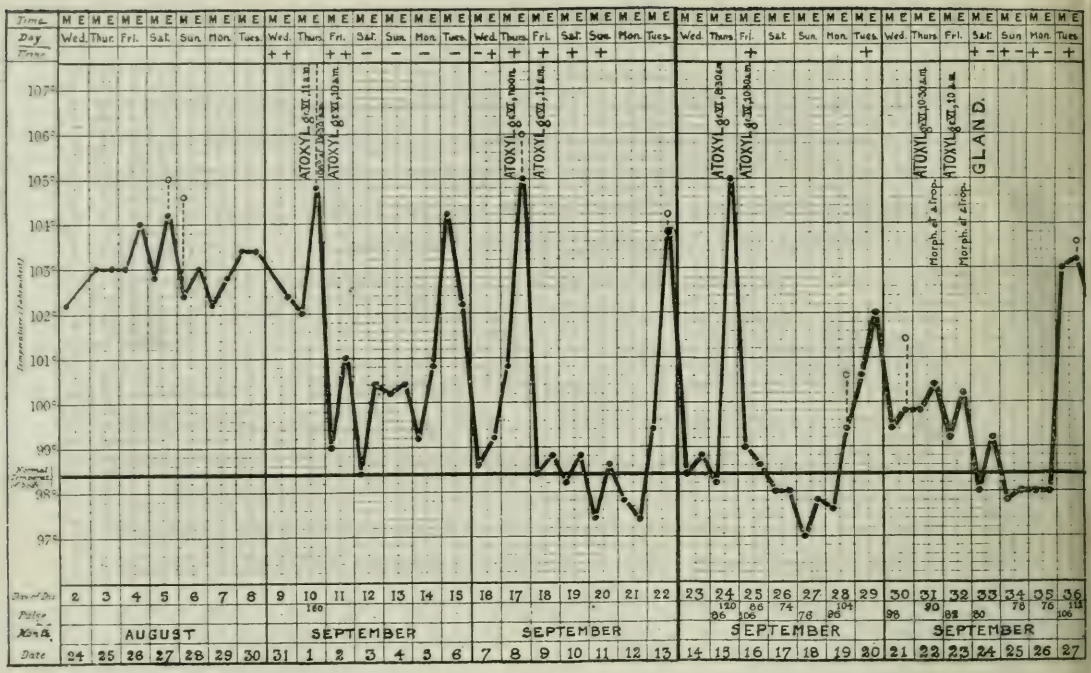
DISEASE.
RYPANOSOMIASIS.

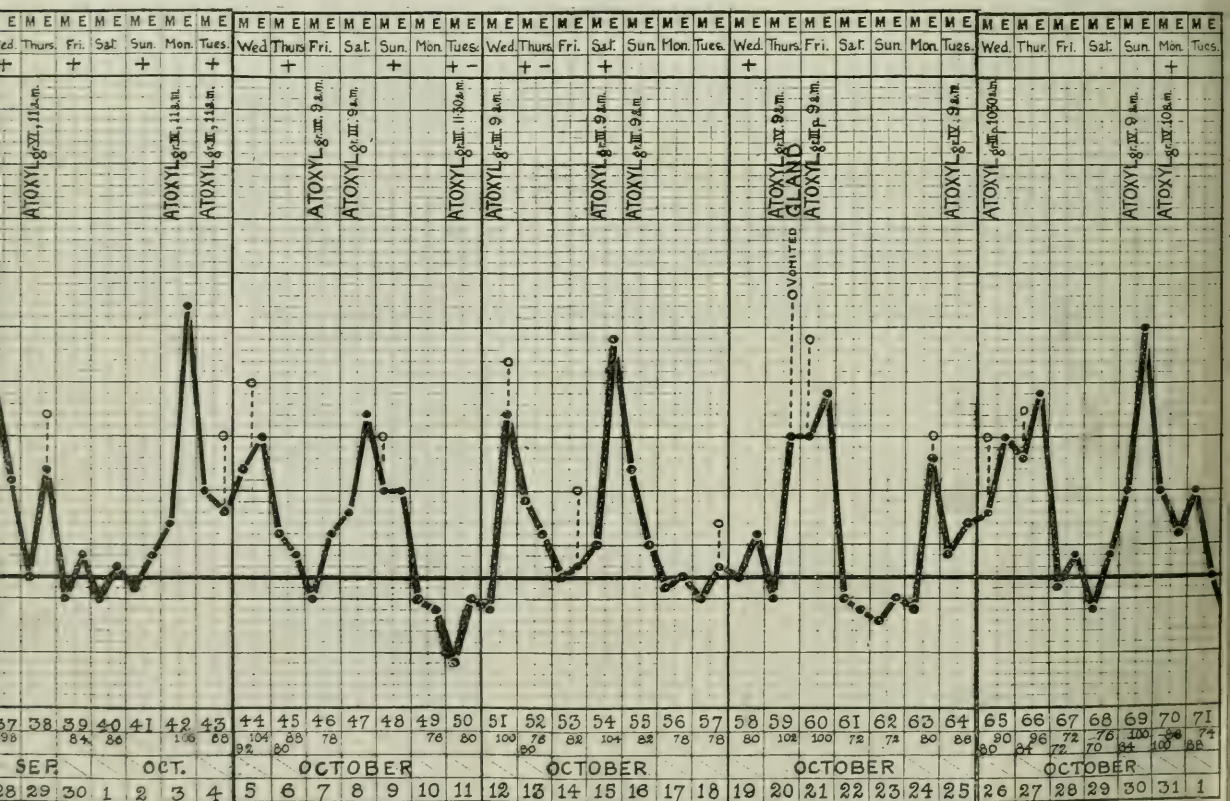
Notes of Case

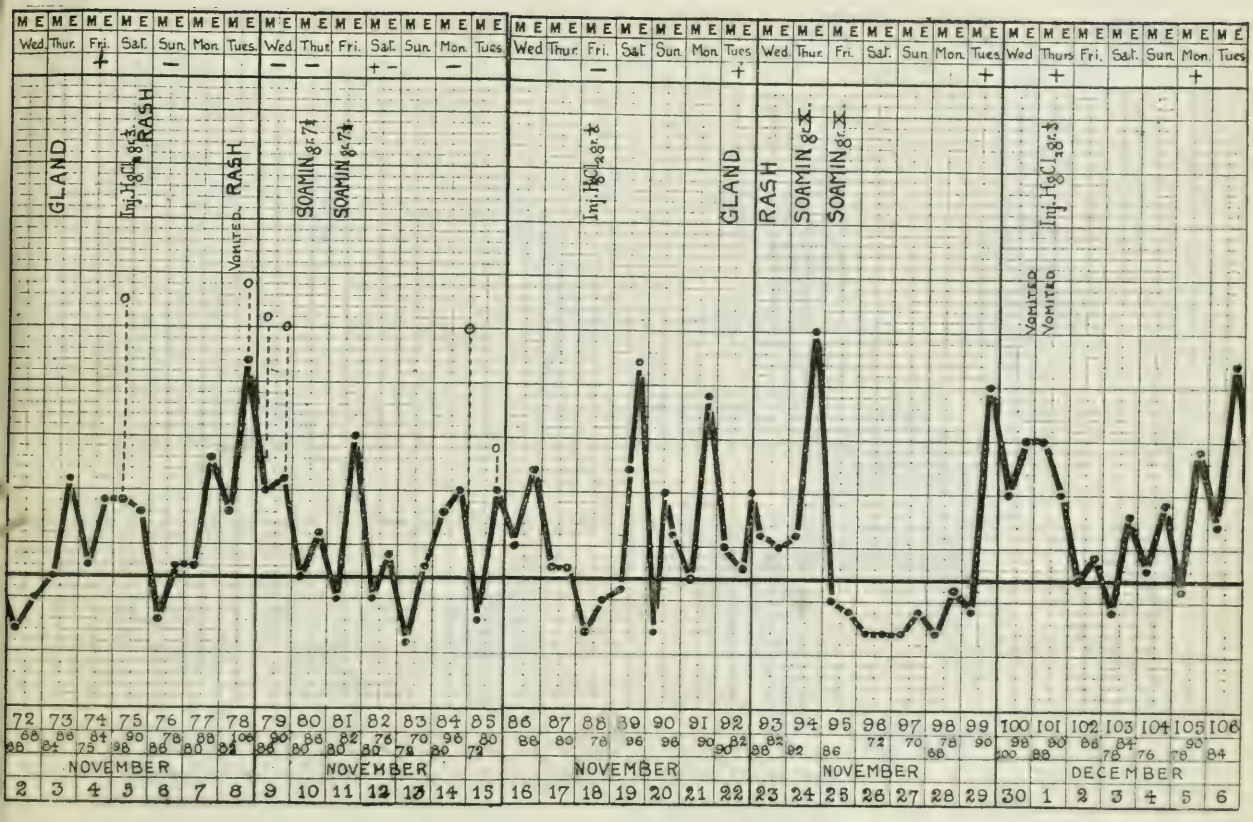
Mr. R.
60.

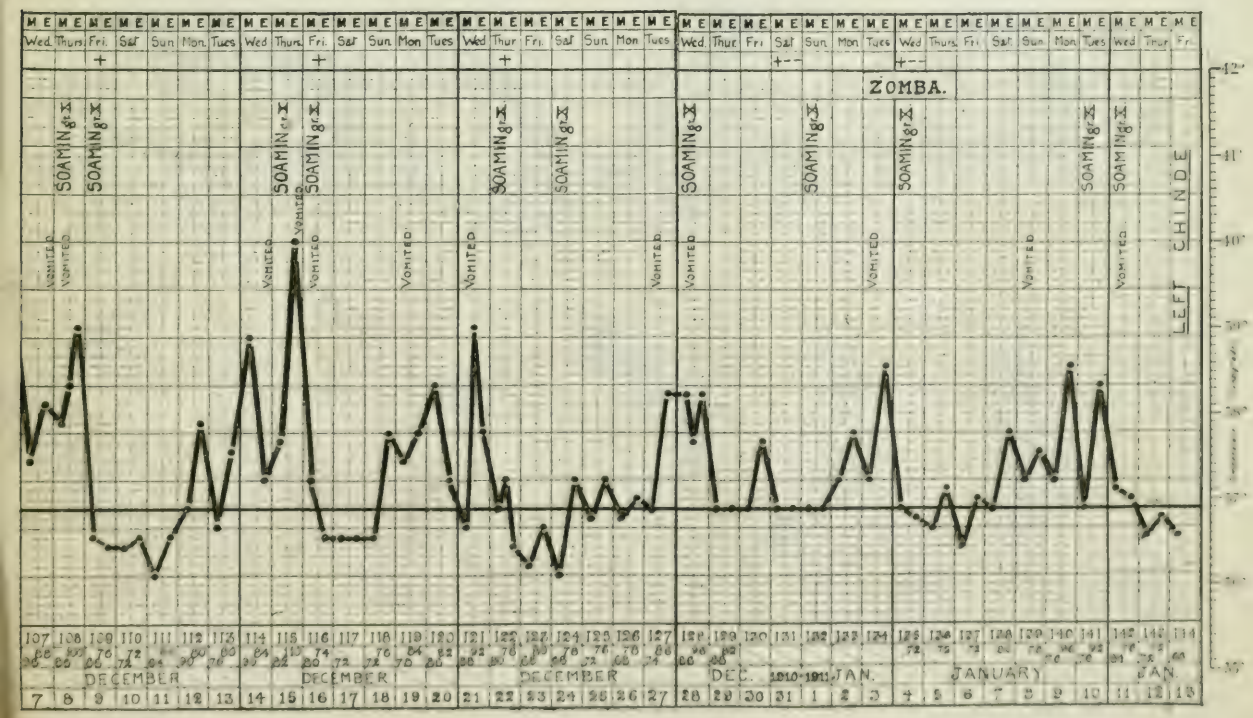
Date of admission

sult






GLAND
Inj. HgCl 1/8 gr.
RASH
VOMITED
RASH
SOAMIN gr. 7½
SOAMIN gr. 7½
Inj. HgCl 1/8 gr.
GLAND
RASH
SOAMIN gr. X
SOAMIN gr. X
VOMITED
VOMITED
Inj. HgCl 1/8 gr.
NOVEMBER
NOVEMBER
NOVEMBER
NOVEMBER
DECEMBER


SOAMIN gr. X
SOAMIN gr. X
SOAMIN gr. X
SOAMIN gr. X
SOAMIN gr. X
SOAMIN gr. X
SOAMIN gr. X
SOAMIN gr. X
SOAMIN gr. X
SOAMIN gr. X
ZOMBA.
LEFT CHINDE
DECEMBER
DECEMBER
DECEMBER
DEC. 1910-1911 JAN.
JANUARY
JAN.

DESCRIPTION OF PLATE XIX

Drawn with Abbé camera lucida, using 2 mm. apochromatic objective and No. 18 compensating ocular (Zeiss). Magnification 2150 diameters.

Figures drawn from parasites in the blood of the monkey, except when otherwise stated.

Figs. 1-4. Forms with the nucleus median. Figs. 1 and 2 show line connecting blepharoplast with nucleus; in figs. 3 and 4 marked vacuolation of cytoplasm is seen.

Figs. 5-12. Forms in which the nucleus is seen to become gradually more posterior until it lies on a level with the centrosome (fig. 5 from patient's blood, fig. 8 from rabbit's blood).

Fig. 13. Division form with nucleus median (from patient's blood).

Figs. 14-17. Division forms with one or both nuclei posterior (figs. 16 and 17 from patient's blood).



A SECOND SERIES OF EXPERIMENTS DEALING WITH THE TRANSMISSION OF GOITRE FROM MAN TO ANIMALS

BY

ROBERT McCARRISON, M.D., R.U.I., M.R.C.P. (LOND.);

CAPTAIN, INDIAN MEDICAL SERVICE,
AGENCY SURGEON, GILGIT, KASHMERE

(*Received for publication* 21 *October*, 1911)

OBJECT OF THE RESEARCH

In a former series of experiments, the results of which were communicated to the Royal Society in June (1911), and published in full in the 'Annals of Tropical Medicine and Parasitology' (1911), it was shown that the thyroid glands of goats, fed on water infected with the faeces of goitrous individuals, underwent in 50 per cent. of cases an increase in size and presented the following changes on microscopical examination:—

(1) A marked dilatation of the vesicles with colloid substance, and a thinning of their walls.

(2) A flattening of the epithelial lining of the vesicles.

The object of the present research was:—

(1) To repeat the experiment of feeding goats on a water highly contaminated with the faeces of goitrous individuals.

(2) To ascertain the effect on the thyroid glands of animals of cultures of micro-organisms grown from the faeces of goitrous individuals, when administered by the mouth.

(3) To ascertain the effect on the thyroid glands of animals of such cultures of micro-organisms when administered by the mouth in association with an artificial water containing the carbonates of lime, magnesium and sodium.

(4) To ascertain the effect on the thyroid glands of animals of the above-mentioned carbonates when administered alone.

The experiments were carried out during the months of January to May, 1911. The animals employed were goats and dogs.

EXPERIMENT A.—The administration to goats of water contaminated with the faeces of goitrous individuals.

The method of preparing this water has been detailed in a former communication[2]: a pure water was allowed to percolate through a mixture of sterilized soil and faeces, and collected in a stoppered bottle. Twelve female goats, aged two years, were confined in a pen on a goitre-free soil, and provided with this fouled water as the only drinking water for a period of 107 days (from 20th January to 6th May, 1911). The animals were liberally fed on a ration consisting of one pound of grain and as much lucerne grass as they could eat. All other possible sources of infection were rigidly excluded. It had been observed in my former experiments that the goats employed lost in weight; I, consequently, added the grain to the ration in the case of the animals of the present experiment. At the end of 107 days seven of the twelve goats were taken at random and killed. Their thyroid glands were removed, weighed, and subsequently examined microscopically. Manual examination of the thyroids of these animals during the course of the experiment showed that in about 50 per cent. of cases the glands had increased in size, and were readily palpable. But it was observed that the size of the organ fluctuated considerably; at one examination it was easily felt and its whole outline could be clearly appreciated, while at another it was felt with difficulty.

The following table shows the weights of the thyroid glands as compared with the body weight of the animals:—

No.	Weight of animal	Weight of thyroid	Proportionate weight of thyroid to weight of animal
I	42 lbs.	2·8 gms.	1:7466
II	42 lbs.	3·0 gms.	1:6,999
III	38 lbs.	1·3 gms.	1:14,600
IV	55 lbs.	3·1 gms.	1:8,800
V	45 lbs.	2·5 gms.	1:9,000
VIII	46 lbs.	1·4 gms.	1:16,400
V	28 lbs.	1·7 gms.	1:8,000

The average weight of a normal goat's thyroid gland is, in Gilgit, $\frac{1}{10,000}$th part of the body weight. It will be observed that three of the above animals (Nos. 1, 2 and 7) had thyroid glands considerably larger than normal. The increase in size was not so marked as in the case of my former experiments, where four out of six goats were found to show an increase in size of the organ varying from $\frac{1}{4,500}$th to $\frac{1}{8,000}$th part of the body weight of the animal. This difference may be attributed to the fact that the animals in the former experiments were not so well fed, and, consequently, *lost* in weight, while those of the present series *gained* in weight. It will further be observed that two other goats (Nos. 3 and 6) had thyroid glands considerably less in weight than normal, while these organs in the remaining animals (Nos. 4 and 5) showed no marked deviation from the normal size.

The histological appearances of the glands of these animals varied very considerably:—

Nos. 4 and 5 showed no appreciable deviation from the normal type. A small area of parathyroid-like tissue was seen in the thyroid of No. 5.

Nos. 3 and 6 differed from the normal type in so far that the vesicles were somewhat smaller and the cells lining the vesicles were higher (low columnar) than is the case in the normal gland.

Nos. 1 and 2 were identical in appearance. The vesicles (fig. 3) were on the whole larger, more irregular in shape, and the total amount of colloid was greater than in the case of the normal gland (fig. 1). The epithelium lining the vesicles was somewhat higher (fig. 4) than in the normal gland (fig. 2). The thyroid in these cases did not present the same degree of dilatation and irregularity of the vesicles, nor the marked flattening of the epithelium, which was observed in the case of my former experiments (fig. 5) (1911). Indeed, apart from the increase in size of the organs, it cannot be said that the histological appearances differed to any very marked extent from the normal gland.

No. 7.—The histological appearances of this organ revealed a considerable degree of hyperplasia. The vesicles were small, the amount of colloid comparatively scanty, the lining epithelium was columnar and the vessels of the gland were dilated. The gland was

markedly more cellular than any other in the present experiment (figs. 6 and 7).

The net result of this experiment, therefore, is that of seven thyroid glands examined three (or 42 per cent.) were larger than normal as determined by weight. Of these, two showed in their histological appearances no very marked variation from the normal type, while the third showed a considerable degree of hyperplasia. Two other glands were in all respects normal, while the remaining two were considerably smaller than normal, and showed a more cellular structure and a higher type of epithelium lining the vesicles than is normal.

EXPERIMENT B.—The administration to goats of cultures of bacteria grown from the faeces of goitrous individuals:

Four female goats, aged two years, were housed and fed in a manner identical with those in Experiment A. They were provided with a drinking water of known purity, which, as an additional precaution, was boiled. The animals were given cultures of bacteria, grown from the faeces of goitrous individuals The experiments lasted 108 days (20th January to 7th May, 1911). During the first month the animals were given, on alternate days, 48-hour cultures on Musgrave's agar of such organisms as had grown on this medium. At this stage of the experiment no attempt was made to purify the growth. Musgrave's agar tubes were inoculated direct from the faeces, incubated, and sub-cultures from the resultant growth were made on Musgrave's agar. After forty-eight hours' incubation an emulsion of the bacteria present was made in distilled water, mixed with milk, and introduced into the stomach of the animal through a funnel. The contents of one tube of a 48-hour culture was given to each animal every other day. The organisms present in such a culture were mainly of two classes: (1) Organisms of the coli group, and (2) a spore-bearing organism, the characters of which will be subsequently described. At the end of the second month a pure culture of the spore-bearing organism referred to was obtained from the faeces of a horse which was suffering from recent goitre, and from the 61st day of the experiment onward this organism alone was given—one tube of a 48-hour growth to each animal on alternate days. The animals were all killed on the 7th May, their thyroid glands removed and subsequently examined microscopically. The

following table shows the weight of the thyroid gland in each case relative to the body weight of the animal:—

No.	Weight of animal on 20th Jan., in lbs.	Weight on 7th May, in lbs.	Increase in weight, in lbs.	Weight of thyroid	Proportionate weight of thyroid to body weight of the animal
I	42	54	12	1·0 gm.	1:27,000
II	48	65	17	2·3 gms.	1:13,000
III	37	40	3	2·0 gms.	1:10,000
IV	32	41	9	1·6 gms.	1:12,000

It will be observed from the table:—

(1) That all the animals increased in weight. In case No. 2 the increase in weight is very marked. In case No. 3, in which the thyroid is of normal weight, the animal increased only three pounds in weight.

(2) That in three cases out of four the thyroid gland was considerably smaller than normal.

There was, therefore, in these animals no evidence of any enlargement of the thyroid gland (goitre). But well-marked histological changes were observed in all animals in this experiment in which the thyroid showed any marked deviation from the normal weight.

No. 1.—The animal increased 12 lbs. in weight. The thyroid gland was almost three times smaller than normal. The following changes were observed on microscopical examination: the vesicles were round or oval, lined with cubical or low columnar epithelium; the colloid was small in amount, areas of parathyroid-like tissue were seen, wholly cellular, showing an absence of colloid, and merging into the vesicular structure of the rest of the gland; this cellular structure was observed to form about one-half of the total area of the section; the capillaries and vessels of the stroma were dilated; there was an increase of the connective tissue stroma of the organ, especially around the blood vessels, the walls of which appeared somewhat thickened.

No. 2.—The animal increased 17 lbs. in weight. The thyroid gland was smaller than normal. The following changes were observed on microscopical examination: vesicles were almost wholly absent or were filled with round, imperfectly-staining cells and cellular débris. Where vesicles were seen they were lined with irregular, high columnar epithelium, the lining being often incomplete in parts. Stainable colloid was wholly absent. The stroma was increased markedly in amount and formed a network, the meshes of which were filled with round cells, many of which stained imperfectly, and cellular débris. The capillaries of the organ were not noticeably altered. The large central artery appeared dilated, though its walls were not thickened (figs. 8 and 9). The appearance of this organ and the great increase in weight of the animal are suggestive of a commencing myxoedema.

No. 3.—The animal increased 3 lbs. in weight during the experiment. The thyroid gland was of normal weight. The histological features of the organ were normal. There was a small circumscribed area of parathyroid-like tissue in the centre of the gland.

No. 4.—The animal increased 9 lbs. in weight during the course of experiment. Microscopical examination of the thyroid gland showed it to be rich in colloid, the vesicles rather larger and more irregular than normal. A small cyst was present. No other abnormalities were noted.

The net result of this experiment, therefore, is that of four goats three had thyroid glands smaller than normal, in two of which histological changes were marked. These changes were in one case those of an active hyperplasia, while in the other they amounted to a fibrosis and cellular degeneration of the organ. The fourth gland was in all respects normal.

Experiment C.—The administration to goats of cultures of bacteria, grown from the faeces of goitrous individuals, together with a known quantity of the carbonates of lime, magnesium and sodium.

The conditions of this experiment were identical with those of Experiment B. The same cultures were employed and administered for the same length of time and in the same way. But one hour previous to the administration of the cultures the four female goats employed in the experiment were given a solution of 5 grains each of

the carbonates of lime, magnesium, and sodium. My object in giving the animals these salts was to ascertain whether they exerted any influence in favouring the development of a goitre in the animals to which the bacterial cultures were being given. In certain localities where the drinking water contains large quantities of lime and magnesium, individual goitres are, as a rule, larger than in other localities where the water is less hard, and though these metals are now known not to cause the disease, yet it is possible they may exert an influence of a secondary importance in the production of the malady. In the present experiment it was thought that the administration of these carbonates might, by increasing the alkalinity of the intestinal contents, favour the development of the bacteria administered by the mouth, these bacteria having been cultivated on an alkaline medium. The experiment was carried out concurrently with the previous one, and lasted the same length of time.

Three of the four goats were killed, their thyroid glands removed and weighed, and subsequently examined microscopically. The following table shows in each case the weight of the thyroid gland relative to the body weight of the animal:—

No.	Weight of animal on 20th Jan., in lbs.	Weight of animal on 7th May, in lbs.	Increase in weight in lbs.	Weight of thyroid	Proportionate weight of thyroid to body weight of the animal
I	44½	55	10½	1·5 gms.	1:18,200
II	41	45	14	1·45 gms.	1:16,000
III	37½	45	7½	2·1 gms.	1:10,630

The histological appearances of the glands of these animals were as follows:—

No. 1.—There was an increase in the connective tissue stroma; colloid was scanty; vesicles were rounded or oval, and either filled with cells or lined with a high columnar epithelium. The vessels of the stroma were not noticeably hypertrophied, though some were dilated. These features indicated a well-marked hyperplasia (figs. 10 and 11).

No. 2.—The microscopical appearances were the same as in the

previous case, but the vesicles were larger, and the cells lining them of a lower columnar type, while the colloid was rather more plentiful.

No. 3.—The gland showed little deviation from the normal type. The colloid was plentiful. The vesicles were here and there lined with a low columnar epithelium. An area of the parathyroid-like tissue was present. Serial sections in this, as well as in other cases, showed that the cellular parathyroid-like area gradually merged into the vesicular structure of thyroid tissue (fig. 12), from which it appeared to differ in no essential, except in the absence of formed vesicles and colloid.

The net result of this experiment, therefore, is that of three goats two had thyroid glands which showed marked degrees of hyperplasia, while the glands were considerably smaller than normal All these animals increased considerably in weight under the conditions of the experiment.

EXPERIMENT D.—The administration to goats of the carbonates of calcium, magnesium and sodium.

In this experiment four female goats of the same age as those in the preceding experiments were employed. The conditions of the experiment were in all respects identical with those of B and C, except that in this case the animals were given only the carbonates of magnesium, calcium, and sodium in doses of five grains of each of these salts every other day. The experiment was carried out concurrently with the preceding one, and was intended mainly as a control to it. On the expiration of 107 days two of the four goats were taken at random and killed, their thyroids removed, weighed, and subsequently examined microscopically. The following table shows in each case the weight of the thyroid gland relative to the body weight of the animal:—

No.	Weight of animal on 20th Jan., in lbs.	Weight of animal on 7th May, in lbs.	Increase or decrease in in weight in lbs.	Weight of thyroid in gms.	Proportionate weight of thyroid to body weight of animal
I	27½	25	— 2½	1·5 gms.	1:8,400
II	37½	43	+ 5½	2·2 gms.	1:9,700

The histological appearances of the thyroid glands of these animals were as follows:—

No. 1.—No deviation from normal could be observed.

No. 2.—In this case the colloid was comparatively scanty, the vesicles were small or oval, and lined with low columnar epithelium. The stroma around the vessels was increased, though not markedly so, around individual vesicles, and stretched into the gland, giving it under a low power a lobated appearance. The vessels were not noticeably dilated or hypertrophied.

The net result of this experiment, therefore, is that one of two goats showed a slight degree of hyperplasia of the thyroid gland.

SUMMARY OF RESULTS

If the results of these four experiments are compared, several broad differences will be noted:—

(1) In those animals which drank only highly faecal polluted water for over three months there was a tendency on the part of the thyroid gland to be larger than normal (3 cases out of 7).

(2) In those animals which were fed on cultures of bacteria from the intestines of goitrous individuals there was a tendency on the part of the thyroid gland to be smaller than normal, and this tendency appears to be well marked (5 cases out of 7). The diminution in size of the thyroid of these animals appears also to be associated with an increase in their body weight.

(3) In those animals which drank a highly faecal polluted water the histological appearances of the gland either differed in no essential from normal, or there was evidence of an increase in size of the vesicles, of irregularity in their shape, of a higher type of epithelium lining the vesicle, and of a total increase in the amount of colloid present.

(4) In those animals which were fed on cultures of bacteria from the intestines of goitrous individuals, a marked tendency to hyperplasia was observed. The cells lining the vesicles were in a large proportion of the cases columnar in type, colloid was scanty, and there was evidence of an increase in the connective tissue stroma of the organ. In one case the stroma was so markedly increased, and the cells so altered as to give rise to the suggestion of commencing myxoedema.

(5) A slight hyperplasia was also observed in one of two goats to which only carbonates of magnesium, lime and sodium had been given.

It appears, therefore, that a considerable hyperplasia of the thyroid gland may occur under various conditions, as it is present in one or more cases in each of the foregoing experiments. But so marked are the histological changes in some of the thyroid glands of the goats of experiments B and C, and so striking is the contrast between them and the glands of normal animals, and of the goats of the other experiments, A and D, that one is led to attribute these changes to the action of the bacteria administered. The cases are, however, too few to admit of more than this general conclusion being drawn; and this conclusion must be subjected to the test of further experiment on a much larger number of animals than were employed in the present instance.

The results of feeding goats on faecal polluted water was in the present series not so marked as in my former experiment. But here also three goats out of seven showed an enlargement of the thyroid gland, as determined by weight. The structure of these glands, however, did not show the same degree of dilatation and distension of the vesicles with colloid, nor was the thinning and irregularity of the walls of the vesicles so marked, or the epithelial lining so flattened as in the thyroid glands of the goats of my first series of experiments. The results, nevertheless, are on the whole similar to those obtained in my former experiments.[1,2]

I have alluded to a spore-bearing bacillus, which was isolated in pure cultures from the faeces of a goitrous horse, and which was constantly present in the cultures from the faeces of goitrous individuals, as having been employed in experiments B and C. I am indebted to the Director of the Pasteur Institute of Kasauli and to the Assistant Director for the following description of this organism. It is a rod-shaped bacillus, varying from 2 to 4 μ in size, which does not retain the stain by Gram's method, but stains well with Carbo-Fuchsin, Leishman's, and other stains. The bacilli vary in size and thickness, and some of them contain a lighter unstained area, situated usually at the centre, but sometimes towards the periphery of the organism. The growth on Musgrave's agar shows, in addition to the bacilli, numerous round unstained bodies, which are seen to be

spores when special staining methods are employed. In some of the bacilli spore formation is also seen. The organism is very actively motile in young cultures. Plate cultures on agar and Musgrave's medium appear as small, round and opaque white colonies. The deeper colonies are irregular, with crenated margins, and are bluish white in colour. The growth is more profuse on alkaline (÷ 1) agar than on Musgrave's medium. On agar slopes (÷ 1) a profuse opaque growth, white lead in colour when viewed from the surface, and faintly brown in colour with transmitted light, is seen after twenty-four hours.

The organism ferments glucose, laevulose, mannose, and galactose, but not dextrose, mannite, lactose or maltose. It produces no curdling or acid in litmus milk, and grows profusely in broth, forming a white scum on the surface. It does not liquefy gelatine in stab cultures; in this medium it forms a button-like growth into the medium, with both superficial and deep gas production. On potato there is a profuse brownish growth. The organism is not killed by 0·5 per cent. carbolic acid for twenty-four hours in an incubator, nor by 60° C. for half an hour. It is not destroyed by boiling at 94° C. for five minutes. Half to one c.c. of a living culture injected into guinea-pigs produced no immediate ill effects. Larger quantities of the living culture injected into dogs, kids, and goats gave a similar result.

A young dog, weighing 9 lbs. 4 ozs., was given nine Musgrave's agar tubes of a forty-eight hour culture of this organism. Two hours later the dog was observed to be making violent efforts to vomit, but without result. About six hours later the animal became stiff and unsteady on his limbs. On the following day he was observed to have developed pronounced clonic spasms of the muscles of the limbs and tail. He was then unable to stand, and when propped up his legs gave way under him. These symptoms persisted, the animal lost consciousness, a blood-stained discharge from the mouth, nose, and anus appeared on the fourth day, and the animal died on the ninth day of the experiment. The thyroid lobes were removed, with two enlarged lymph glands in their vicinity. Two agar (÷ 1) tubes were inoculated with the blood-stained fluid which escaped from the cut surface of the lymph glands. After twenty-four hours' incubation several colonies of the spore-bearing organism above described were

present in one of the tubes. The other tube remained sterile. The thyroid gland of the animal was about one-third of the size of another dog, which weighed 10 lbs. 0 ozs., and which was killed for purposes of comparison. The organ on microscopical examination was found to be almost wholly cellular, with an occasional vesicle of normal appearance scattered here and there through the section. The connective tissue stroma appeared to be increased, and formed a well-marked network, the meshes of which were filled with round cells, and with no attempt at the formation of vesicles. Colloid was wholly absent except in the few scattered vesicles. The central artery of the gland appeared considerably thickened. The appearances seen are shown in fig. 13, magnified about 500 diameters. Fig. 14 shows the thyroid of the 'bazaar' dog which was killed for purposes of comparison. The magnification is in both cases the same.

Two other dogs were subsequently given the same number of tubes of a forty-eight hour culture of this organism on Musgrave's medium, but the animals remained to all appearances quite healthy. They were, unfortunately, not killed.

If the micro-photograph of this animal's thyroid is compared with that of goat No. 2 B (figs. 8 and 9), which was fed on cultures of this organism for a period of two months, a striking resemblance will be observed in the two cases. There is the same increase of fibrous tissue, the same cellular structure of the gland, broken up into columns of rounded cells by the network of stroma, the same absence of colloid, complete in the case of the goat, partial and limited to a few scattered vesicles in the case of the dog. Fig. 13 also resembles very closely that of a dog's thyroid figured by Mr. Edmunds in his work on: 'The Pathology and Diseases of the Thyroid Gland'[3] (fig. 19, p. 36); this figure shows the changes in a dog's thyroid 'four days after partial removal.' Mr. Edmunds, in speaking of the changes which take place in the thyroid as a result of removal of the parathyroids, refers to this particular case as one in which, so far from the thyroid increasing in size, it appeared to have been smaller than normal. The small size of this dog's thyroid and of the goats' thyroids in my experiments B and C is very striking

That the experiments detailed in this paper have yielded striking results there is no doubt, but I do not at this stage of my observations propose to do more than record them. The number of animals

employed was too small to permit of definite conclusions being drawn, but the results here recorded are sufficiently marked to justify the belief that experiments conducted on a larger scale, and on the same lines, would yield valuable information. In connection with these experiments, I would draw attention to a paper which I read before the Royal Society of Medicine on the subject of the 'Vaccine Treatment of Goitre,' and in which I detail the results of treatment of incipient goitre by vaccines prepared from coliform bacilli, staphylococcus, and the spore-bearing organism described in this paper.

REFERENCES

1. McCarrison, R. (1911). Proc. R. S., B, Aug. 18, LXXXIV, 570, pp. 155-156.
2. ——— ,, Ann. Trop. Med. & Parasitol., Aug. 1, V, 2, pp. 187-198.
3. Edmunds, W. The Pathology and Diseases of the Thyroid Gland.

DESCRIPTION OF PLATES

PLATE XX

Fig. 1. Thyroid gland of normal control goat. × 100.

Fig. 2. Thyroid gland of normal control goat. × 500.

Fig. 3. Thyroid gland of goat No. 2 (Experiment A). The vesicles are more irregular in shape and the total amount of colloid is greater than in the normal gland. × 100.

Fig. 4. Thyroid gland of the same animal magnified 500 times. The micro-photograph shows the more irregular shape of the vesicles and the higher type of the epithelium lining the vesicles than in the case of the normal gland.

Fig. 5. Thyroid gland of goat, showing dilatation and irregularity in shape of vesicles, thinning of vesicular walls, and increase in the amount of colloid. From a case of artificially-produced thyroid enlargement in a goat (1911). × 700.

Fig. 6. Thyroid gland of goat No. 7 (Experiment A), showing scanty amount of colloid, columnar epithelium lining the vesicles, and dilatation of vessels. × 100.

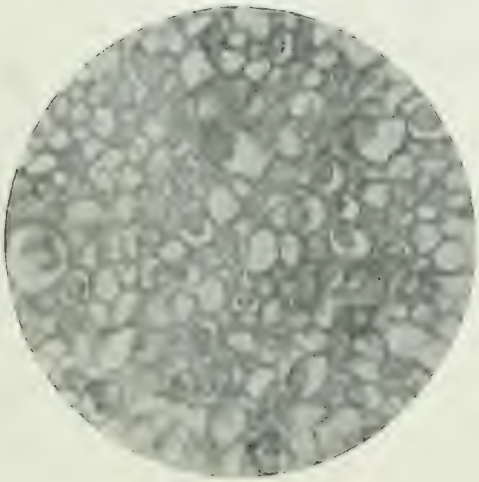


FIG. 1 × 100.



FIG. 2 × 500.



FIG. 3 × 100.



FIG. 4 × 500.

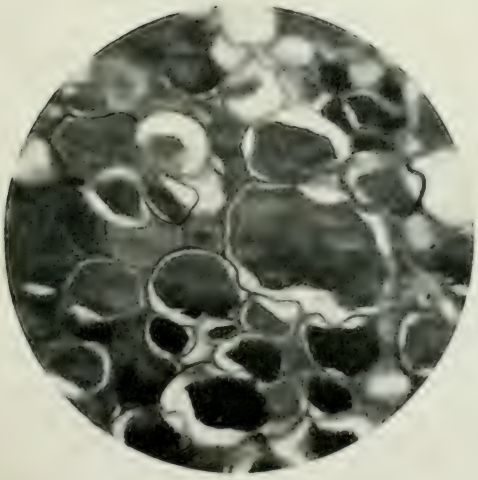


FIG. 5 × 100.

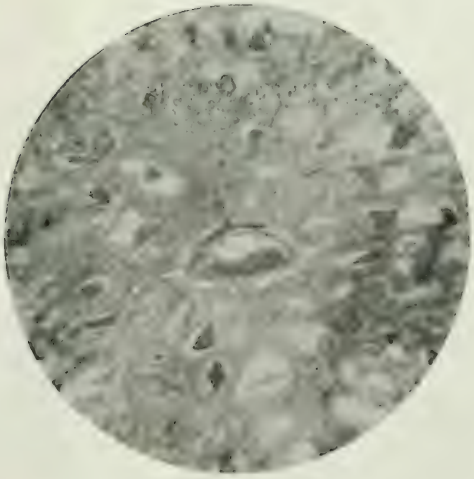


FIG. 6 × 100.

P. P. Press, Imp.

Plate XXI

Fig. 7. Thyroid gland of the same animal magnified 500 times; showing the columnar epithelial lining of the vesicles, and the dilated vessels in the lower part of the field.

Fig. 8. Thyroid gland of goat No. 2 (Experiment B), showing almost complete absence of vesicles and colloid. × 100.

Fig. 9. Thyroid gland of the same animal magnified 500 times, showing the increase of the fibrous stroma and the masses of cells filling the meshes of the stroma, also the complete absence of colloid.

Fig. 10. Thyroid gland of goat No. 1 (Experiment C), showing vesicles lined with high columnar epithelium, and the scanty amount of colloid. × 100.

Fig. 11. Thyroid gland of the same animal magnified 500 times, showing the high columnar epithelium lining the vesicles.

Fig. 12. Thyroid gland of goat No. 3 (Experiment C), showing area of parathyroid-like tissue in lower left quadrant of field. × 100.



FIG. 7 × 500.

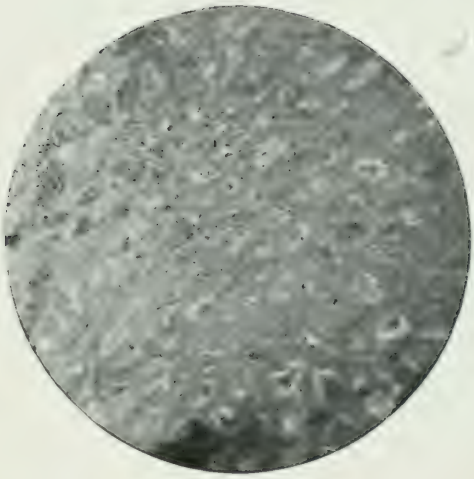


FIG. 8 × 100.

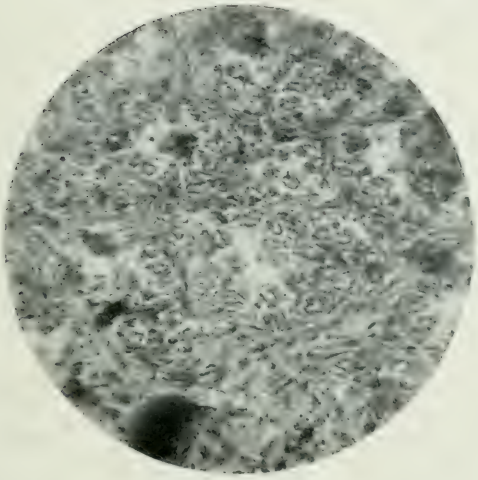


FIG. 9 × 100.



FIG. 10 × 100.



FIG. 11 × 500.

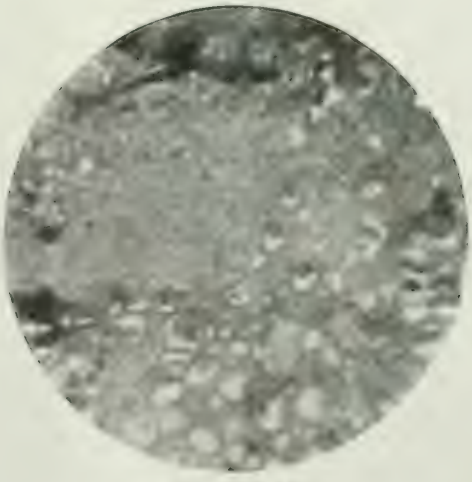


FIG. 12 × 100.

P. P. Press, Imp.

PLATE XXII

Fig. 13 Thyroid gland of a dog, showing the cellular structure of the organ, and the absence of vesicles and of colloid. × 500.

Fig. 14. Thyroid gland of normal 'bazaar' dog, showing the normal appearance of the thyroid gland of a dog. × 500.



FIG. 13 × 500.

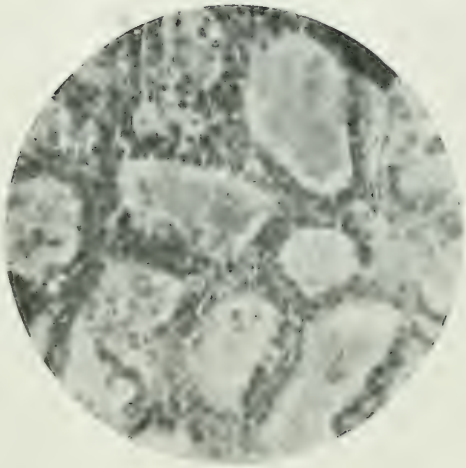


FIG. 14 × 500.

P. P. Press, Imp.

A NEW BLOOD-COUNTING PIPETTE, FOR ESTIMATING THE NUMBERS OF LEUCOCYTES AND BLOOD PARASITES PER CUBIC MILLIMETRE

BY

DAVID THOMSON, M.B., CH.B. (EDIN.) D.P.H.(CAMB.)

(*Received for publication* 8 *November*, 1911)

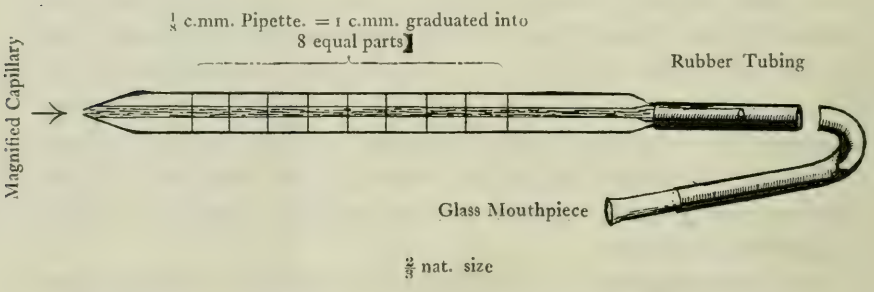
Prefatory Note.—This pipette* has been devised to facilitate an extensive research, carried on during the past two years, on the exact enumeration of parasites in the blood of cases of malarial fever and sleeping sickness. The research was instigated and directed by Sir Ronald Ross, K.C.B., F.R.S., and conducted with the aid of funds supplied by the Advisory Committee of the Colonial Office and a private fund given by Sir Edwin Durning-Lawrence in connection with cryotherapy, at the Liverpool School of Tropical Medicine, and at the Tropical Laboratory in the Royal Southern Hospital, Liverpool, and has revealed a number of facts in both these diseases.

We first attempted to enumerate the parasites in malarial fever by finding the ratio of leucocytes to parasites in dehaemoglobinised thick blood films (Ross, 1903). This method, however, proved to be tedious and inaccurate, as it involved the double process of making a leucocyte count with a Thoma Zeiss, and finding the leucocyte-parasite ratio in a thick blood preparation. Later, at the suggestion of Sir Ronald Ross, I commenced to count the parasites directly in a measured sample of blood, blown from a graduated capillary pipette and made into a thick film. This latter method gave more accurate results, and, finally, by devising a special pipette, I was able to enumerate quickly and with considerable accuracy both leucocytes and parasites simultaneously in the specimen. The references to the literature of the researches carried on by this method will be found at the end of this paper.

* The pipettes are sold by C. Baker, 244 High Holborn, London, W.C., Price 10/6 each.

I. DESCRIPTION OF THE PIPETTE

The pipette is about five inches long, and is made of glass tubing similar to that used for thermometers, with a white opal background and powerful magnifying surface. The capillary bore, however, is not flattened out like that of a thermometer but is more nearly circular in cross section. The bore is exceedingly fine and hair-like—such that a portion of the capillary about 60 to 70 millimetres long has a capacity of one cubic millimetre. This cubic millimetre length is graduated into equal parts, so that a given fraction of a cubic millimetre of blood can be accurately blown out. A rubber tube with a glass mouthpiece is attached to one end of the pipette, and the other end tapers to a very fine point—which is necessary for expelling accurate small droplets of blood (see diagram).



The pipette possesses about the limit of capillary fineness for blood work, because in using a capillary of less diameter it is difficult to expel the blood (which clots quickly in so fine a bore). When this clotting occurs it is troublesome to clear the pipette, as there is no wire fine enough or rigid enough to penetrate it. Even with the bore employed, considerable care and practice are required; but in experienced hands the pipette may be used constantly for months without any blockage occurring. In view of the above difficulty, I have devised a similar pipette with a larger bore and graduated to $\frac{1}{4}$ c.mm. This latter can be easily penetrated by a fine wire and cleaned in case of a stoppage. Such a pipette (of the larger bore) may be preferred by beginners; but for quicker work and for those who have had some practice, the $\frac{1}{8}$ c.mm. pipette is the better.

II. METHOD OF USING THE PIPETTE

(1) Prick the ear or finger, and allow a tiny droplet of blood to exude. Do not squeeze (or only very little), as squeezing drives out lymph and lymphocytes from the lymphatics.

(2) Suck the blood into the pipette.

(3) Expel the blood until the column coincides with a line on the magnifying surface. Wipe the point of the pipette and expel $\frac{1}{8}$ or $\frac{1}{4}$ c.mm. of blood on to a clean glass slide. After this has been carefully done, expel all the blood immediately from the pipette, otherwise it will clot in the bore.

(4) Breathe on the measured droplet of blood on the slide, and spread it by means of the point of the pricking needle into a small square, about 4 mm. × 4 mm. This square should be spread as uniformly and as neatly as possible, and takes a little practice. A fine triangular pointed needle serves the purpose best. A certain amount of breathing on the slide is essential to keep the blood from drying up during the spreading process. In the case of a $\frac{1}{4}$ c.mm. droplet, the spread square should be a little larger—about 5 mm. × 5 mm.

(5) Allow the little square blood film to dry in air. This takes place in a few seconds. Fix for about two minutes in absolute alcohol and stain with the usual blood stains. In the case of Jenner's or Leishman's stain, previous fixing is of course unnecessary. Wash gently and dry on blotting paper.

(6) If it is desired to enumerate asexual malaria parasites, the square film should be spread over a smaller area into a thicker film and fixed in acid alcohol (5 per cent. dilute acetic acid in absolute alcohol) before staining. The corpuscles are dehaemoglobinised by this procedure and do not obscure the parasites. To count leucocytes, trypanosomes and crescents this dehaemoglobinisation is unnecessary.

(7) The pipette should be cleaned and dried immediately after use. This is done by sucking up water and expelling it alternately several times. Repeat this process with absolute alcohol or ether, and finally suck air through to dry the bore. By watching the bore through the magnifying surface one can tell when it is dry. The water and alcohol used for cleansing and drying should be free from dust.

III. HOW TO CLEAN THE PIPETTE IN CASE OF STOPPAGE

If the blood should clot in the pipette, it can often be expelled by moistening the point of the pipette with water or by forcing water through with a Higginson's syringe. If this fails it may be cleaned as follows. Heat the pipette in the Bunsen flame gradually, but do not heat the point. Now immerse the point in strong nitric acid, and allow to stand there till it cools. This enables the nitric acid to get into the bore. Dry the outside of the pipette; press a piece of indiarubber firmly against the point and at the same time heat it with the Bunsen flame as near to the point as possible. This procedure produces vaporised nitric acid in the bore under high pressure. The vapour eats away the clot and drives it upwards. The process may have to be repeated several times if the clot is a large one. The clot may also be slowly dissolved by completely immersing the pipette in a test-tubeful of nitric acid. Place the test-tube in a beaker of water and alternately boil and cool. This latter is a slower but safer cleansing procedure. In the first method the pipette must be dry and heated gradually, otherwise it may crack.

Sometimes the point only of the pipette becomes blocked with material other than blood. It can easily be cleared by keeping the point immersed in strong nitric acid for some time.

When the pipette is in constant use, it is a good practice to suck strong nitric acid into the bore and allow it to remain there all night. This cleans away any blood or serum which may have commenced to accumulate in the bore or at the orifice. Before using the pipette one should blow through it into alcohol to see if the air bubbles through freely; as the most frequent cause of blocking is an attempt to suck up and expel blood through a partially obstructed orifice. The $\frac{1}{4}$ c.mm. pipette can be cleaned out by means of a fine wire.*

IV. METHOD OF ENUMERATING LEUCOCYTES OR PARASITES IN THE MEASURED SQUARE BLOOD FILM

A microscope with a mechanical sliding stage and an eyepiece having a diaphragm with a square hole is essential. For the latter

---

* For the pepsin method of cleaning pipettes, see Stephens & Christophers, ' Practical Study of Malaria,' third edition, page 11.

a circle of paper with a square hole may be fitted into the eyepiece; but an Ehrlich's ocular eyepiece is better, as by means of this eyepiece the square microscopic field can be contracted by means of a little lever to any size required.

Place a drop of oil on the square blood film, without a cover-glass, and place under oil immersion lens. Find the upper margin of the square film, and by means of the mechanical stage work down, field by field, towards the lower margin. On reaching the fifth field from the upper margin, stop, and move the field from the right margin of the square film to the left margin, meanwhile counting the number of leucocytes or parasites all the way in one imaginary band the breadth of the microscopic field. Now move down to the tenth band from the top margin and repeat this process, then move down to the fifteenth band and repeat again, and finally move down to the lower margin.

Let us suppose that the number of microscopic field diameters between the upper and lower margins is 30, and that the average number of leucocytes or parasites in the three bands counted is 40, then the total number in the square film is $30 \times 40 = 1200$. But the square film represents $\frac{1}{8}$ c.mm., therefore the number per c.mm. is $8 \times 1200 = 9600$. If the square film is $\frac{1}{4}$ c.mm., then the number will be, of course, 4800 per c.mm.

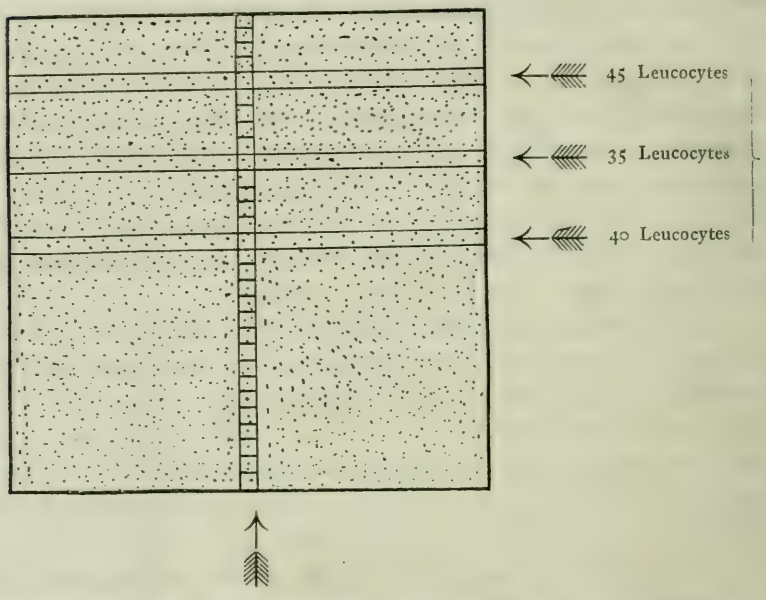
The diagram on page 476 may indicate the method more clearly.

The greater the number of bands counted, the greater is the accuracy of the result; but in a well-spread film where the distribution of leucocytes or parasites is fairly uniform, a count of three bands will be amply sufficient, but one must count a varying number of bands according to the number of parasites or leucocytes present. Where the parasites are scarce, say under 1000 or 2000 per c.mm. (crescents are nearly always below this number), then it is not sufficiently accurate to calculate the number from bands, and the total number in the whole of the square film must be counted by means of the mechanical stage. Where the numbers are very small, it may be necessary to take a larger specimen of blood than $\frac{1}{8}$ or $\frac{1}{4}$ c.mm. The examination of the whole square takes about ten minutes if the objects counted are few.

Special slides can be obtained having a square 4 mm. × 4 mm. ruled on the glass. This enables one to spread the droplet of blood

into an accurate square, and by finding the number of diameters of the microscopic field across this ruled square, one obtains a constant multiplying factor for estimating the number of leucocytes, etc., in blood films spread exactly over that square.

Square Blood Film $\frac{1}{8}$ c.mm.



The side of the Total Square Film is 30 times the diameter of the Square Microscopic Field.

Average number of leucocytes is 40 in a band the breadth of microscopic field.

$\therefore$ $40 \times 30 \times 8 = 9600$ leucocytes per c.mm.

V. SOURCES OF ERROR IN THE METHOD

I do not claim to have calculated the amount of error in this method mathematically, but so far as I can estimate, the errors are as follows:—

(1) With this pipette I estimate that the instrumental error in transferring $\frac{1}{8}$ c.mm. of blood to a slide is 5 per cent.

(2) Where only a few bands are counted across the square, there is an error due to unequal spread of the film. The greater the number of bands counted the smaller is the error, and where the whole of the square is counted this source of error is eliminated altogether.

(3) The error depending upon the number of objects counted has been fully discussed by Sir Ronald Ross and Mr. Walter Stott (1911). By referring to tables in their article, one can tell the exact percentage of error for the number of objects counted. It appears that to get within a statistical error of 5 per cent. one must count at least 200 objects. (For further details see Section IV of their article.)

VI. COMPARISON WITH THE THOMA-ZEISS METHOD

The following table gives a comparison between leucocyte counts made simultaneously by this method and by the Thoma-Zeiss apparatus:—

Case	Thoma-Zeiss	New method (average of two bands only)
1	27,000 leucocytes per c.mm.	26,000 leucocytes per c.mm.
2	15,000 ,, ,,	16,000 ,, ,,
3	14,000 ,, ,,	12,400 ,, ,,
4	16,000 ,, ,,	15,500 ,, ,,
5	13,000 ,, ,,	13,600 ,, ,,
6	40,000 ,, ,,	43,000 ,, ,,
7	10,000 ,, ,,	10,200 ,, ,,
8	17,000 ,, ,,	15,100 ,, ,,

VII. ADVANTAGES OF THE NEW METHOD

(1) Blood parasites can be enumerated. The parasites of malaria and sleeping sickness cannot be enumerated accurately by any other means.

(2) Only a tiny droplet of blood is required. This renders the method very suitable for those who desire to investigate the blood of small animals such as rats, mice, etc. It is also important where frequent examinations are being made on one patient. The patient does not object to the gentle prick of the needle nor to the small amount of blood taken.

(3) No diluting fluid or special slide is required.

(4) The slides with the required square films can be stored,

stained and counted at any future time when convenient. A large number of samples can thus be taken at frequent intervals and these can be kept and examined at a later date.

(5) A differential leucocyte count can be made simultaneously with the enumeration.

(6) Auto-agglutination of the red cells can be detected through the magnifying surface of the capillary.

(7) After some practice the method will be found to be extremely rapid and convenient.

I am indebted to Dr. J. J. Levin who kindly made the Thoma-Zeiss counts in the above table.

REFERENCES TO LITERATURE

Fantham, H. B. (1910). The Life History of *Trypanosoma gambiense* and *Trypanosoma rhodesiense* as seen in Rats and Guinea-pigs. Ann. Trop. Med. & Parasitol., Liverpool, IV, pp. 465-485.

———, & Thomson, J. G. (1910). Enumerative Studies on *Trypanosoma gambiense* and *Trypanosoma rhodesiense* in Rats, Guinea-pigs and Rabbits. Periodic Variations Disclosed. Ibid., IV, pp. 417-463.

Korke, V. T. (1910). Some Observations on a Case of Sleeping Sickness: Coagulation Time of Blood, Albumoses, Choline, Cerebral Sections. Ibid., IV, pp. 325-331.

——— (1911). On the correlation between Trypanosomes, Leucocytes, Coagulation Time, Haemoglobin and Specific Gravity of Blood. Ibid., V, pp. 127-131.

Ross, R. (1903). The Thick Film Process for the Detection of Organisms in the Blood. Thompson-Yates & Johnston Lab. Rep., Liverpool, V, I, pp. 117-118.

———, & Stott (1911). Tables of Statistical Error. Ann. Trop. Med. & Parasitol., Liverpool, V, 3, pp. 347-369.

———, & Thomson, D. (1910). A Case of Sleeping Sickness studied by Precise Enumerative Methods: Regular Periodical increase of the Parasites Disclosed. Ibid., IV, pp. 261-265.

———, & ——— (1910). Some Enumerative Studies on Malarial Fever. Ibid., IV. pp. 267-306.

———, & ——— (1910). A Case of Sleeping Sickness studied by Precise Enumerative Methods: Further Observations. Ibid., IV, pp. 395-416.

———, ———, & Simpson, G. C. E. (1910). A Case of Blackwater Fever followed by a Peculiar Relapse without Haemoglobinuria or Detectable Plasmodia. Ibid., IV, pp. 307-312.

——— & Thomson, J. G. (1910). Experiments on the Treatment of animals infected with Trypanosomes by means of Atoxyl, Vaccines, Cold, X Rays and Leucocytic Extract; Enumerative methods employed. Ibid., IV, pp. 487-527.

Thomson, D. (1911). A Research into the Production, Life and Death of Crescents in Malignant Tertian Malaria, in Treated and Untreated Cases by an Enumerative Method. Ibid., V, pp. 57-82.

——— (1911). The Leucocytes in Malarial Fever: A Method of Diagnosing Malaria long after it is apparently cured. Ibid., V, pp. 83-102.

SOME RESEARCHES ON THE LIFE-CYCLE OF SPIROCHAETES

BY

H. B. FANTHAM, D.Sc., B.A., A.R.C.S.

(*Received for publication* 9 *November*, 1911)

CONTENTS

	Page
Introduction	479
Material and Methods	479
Blood-inhabiting Spirochaetes: *S. recurrentis*, *S. duttoni*, *S. marchouxi* ...	480
Division—longitudinal and transverse	481
Multiple transverse fission in blood of Vertebrates	484
Some observations on *S. duttoni* and *S. marchouxi* in Ticks, young and adult	485
Spirochaetes of Lamellibranchs: *S. balbianii*, *S. anodontae*, *S. solenis* ...	488
Nomenclature	492
Summary	493
Addendum	495
References	496

INTRODUCTION

The elucidation of the complete life-cycle of Spirochaetes is a matter of considerable importance from the scientific and economic point of view. African tick fever and European relapsing fever are due to *Spirochaeta duttoni* and *S. recurrentis* respectively, while *S. marchouxi** has fatal effects in chickens. Spirochaetes may also occur in the digestive tract of many hosts, as is the case in many game birds and in a large number of molluscs. Both blood-inhabiting Spirochaetes and those of Lamellibranchs have claimed my attention for some years, and the following are notes supplementing my previous work, and contributing new items to our knowledge of the life-history of Spirochaetes.

MATERIAL AND METHODS

The work relates to Spirochaetes of the blood, *S. duttoni*, *S. recurrentis* and *S. marchouxi* (= *gallinarum*),* and comparison has been made throughout with the Spirochaetes of Lamellibranchs,

* The Spirochaete of fowls, first described by Marchoux and Salimbeni in 1903, was named *S. marchouxi* by Nuttall in a paper read on Dec. 9, 1904. It was also named *S. gallinarum* by Stephens and Christophers in a book with a preface dated Nov., 1904, but published in 1905. References are given on p. 496.

S. balbianii in *Ostrea edulis* and *Tapes aureus*, *S. anodontae* and *S. solenis* (nov. sp., with pointed ends) from *Solen ensis*.

Many observations have in each case been made on the living organisms, and confirmed later by the examination of stained preparations. For examination of fresh material, use has been made of thermostats and warm stages kept at 37° C. and at 25° C., while preparations have also been examined at room temperature. The paraboloid condenser has been of service throughout, though not indispensable. For staining, iron haematoxylin, Delafield's haematoxylin, gentian violet, thionin and Giemsa's stain have been of most use after wet fixation with osmic acid, corrosive acetic alcohol or Bouin's fluid. Zeiss 1/12″ and 2 mm. objectives with compensating oculars 8 and 12 have been used.

BLOOD-INHABITING SPIROCHAETES

Spirochaeta duttoni, *S. recurrentis* and *S. marchouxi*

These Spirochaetes have long, narrow bodies with many spiral coils. Each has a firm cuticle or periplast from which the protoplasmic



Fig. 1.—Diagram of *S. duttoni*, showing chromatin granules, pointed ends and slight membrane edge.

contents can be squeezed out with much difficulty, leaving the empty periplastic sheath or cuticle behind, as was pointed out by Stephens in 1906. A very tenuous membrane is present (Fig. 1),

being often so closely contracted against the body that it is almost invisible in the living organism and in many stained specimens. The nucleus consists of a series of bars or rodlets ('granules') of chromatin distributed along the body. The structure is most difficult of discernment owing to the minute diameter of the body, but after prolonged staining with Romanowsky solution the body exhibits alternate red areas of chromatin and paler bluish areas of cytoplasm. In life the body appears homogeneous, probably owing to the refractivity of the periplast.

The figures of the structure of *S. marchouxi*, published by Prowazek in 1906, seem to me to be most accurate.

DIVISION

In the case of the above Spirochaetes multiplication in the Vertebrate host is brought about by both longitudinal and transverse fission. Both processes have been repeatedly observed in life, and there has been no confusion of longitudinal division with either entanglement forms or the flexed or 'incurvation' form of transverse division, recently described by Gross (1910) for certain Spirochaetes of *Pecten jacobaeus*.

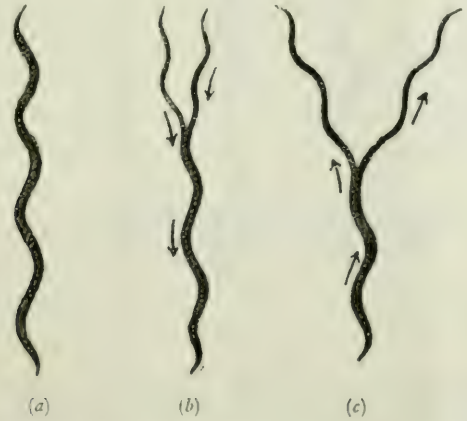


Fig 2.—Diagram illustrating longitudinal division. (*a*) Normal Spirochaete, waves passing alternately in either direction. (*b*) Waves passing from split to undivided end. (*c*) Return waves. Split extending.

True longitudinal division occurs in somewhat thicker individuals found at the beginning of infection and exhibiting a perfectly distinct

clear body, without any entanglement or curvature on themselves. Each organism may be in rapid backward and forward progression a second or so before the onset of division. Rapid waves of contraction followed by relaxation pass down the body of the Spirochaete. A split appears at one end, and gradually widens. The waves pass down each of the daughter forms, which diverge from one another (Fig. 2) until they lie at an angle of 180°, when separation occurs. At the commencement of longitudinal division by no means could a second body, or flexion of one body simulating such, be distinguished, no matter what form of examination were adopted. Longitudinal division is best observed at the onset of infection as recorded by Fantham and Porter in 1909. The resultant forms are half the width of, but the same length as, the body of the parent.

Balfour (1911*b*) has recorded longitudinal division in the Spirochaete (*S. granulosa*) of Sudanese fowls.

Transverse division, in my experience, occurs usually in straight forms. There is no need of looping, 'incurvation,' 'rolling up,'

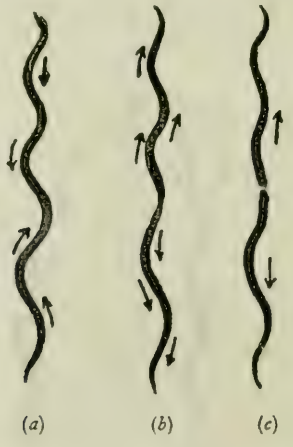


Fig. 3.—Diagram illustrating transverse division. (*a*) Shows waves passing from either end towards a centre or node. (*b*) Shows direction of return waves outwards. Node thinner as result of succession of waves outwards and inwards. (*c*) Daughter Spirochaetes moving away in direction of the outward waves.

or other contortion-figures as a preparation for the act of transverse division. Such contortion may occur, but my experience both with

these blood Spirochaetes and with those of Lamellibranchs is that such movements are but rarely preliminaries to division.

Ordinary straight-lying Spirochaetes, perhaps a little longer than their fellows, divide transversely. Waves pass from each end of the organism towards its centre, where they mutually extinguish one another and induce return waves towards the ends. These processes are repeated many times and the nodal point becomes somewhat thinner, while the newly forming organisms elongate slightly. By a final slight thinning of the node, separation is effected (Fig. 3).

'Delusion' and 'Contortion' division. At various times workers unable to observe longitudinal division of Spirochaetes, partly due to the periodicity of direction of division described by Fantham and Porter in 1909, have thought that the intertwining of two Spirochaetes and their subsequent separation has been mistaken for longitudinal division. Such is not the case. Large numbers of such interlocked forms have been observed and their significance fully realised. In such cases, at some period, two bodies are visible at the 'undivided' end. Further, the series of waves in intertwined forms is modified considerably, and the result is very unlike what is present in true longitudinal division.

Looped or flexed forms of Spirochaetes, which may divide transversely in some cases, have been suggested as possible explanations of longitudinal division. Here again, the two parts of the body of the flexed organism can be detected and also the flexion can be witnessed and recognised for what it is (Fig. 4).



FIG. 4.—Diagram of flexed and intertwined form. Transverse division may sometimes occur at the loop. Usually the organism uncurls and swims away. Alleged by some to be mistaken (!) for true longitudinal division.

Any one who has carefully watched Spirochaetes in life need fall into no such error regarding the nature of the movement. Such flexed or 'incurved' individuals may become slightly thinner and break at the point of flexion, but in my experience this is somewhat rare. The mode of division induced by wave motion has always been the usual method.

Transverse division occurs more particularly when the infection of Spirochaetes is abundant (Fantham and Porter, 1909) and hence is more easy of observation than is longitudinal division.

MULTIPLE TRANSVERSE FISSION OF THE BLOOD SPIROCHAETES WITHIN THE VERTEBRATE HOST

I have observed that a very small number of *S. duttoni*, *S. recurrentis* and *S. marchouxi*, while in the blood of their Vertebrate hosts pass through a peculiar form of asexual multiplication which, for want of a better term, I denote multiple transverse fission. The protoplasmic contents of the Spirochaete concentrate around the chromatin masses forming a number of segments within the periplast which acts as a sheath. A number of small, round or oval bodies ('granules') are thus formed. These may be the diameter of the body of the parent or, if they lie obliquely or curved as they sometimes do, may exceed it very slightly. The effect may be compared with a series of small biconvex or spherical tabloids within a thin skin. The individual small bodies may be compared with cocci. The periplast ultimately ruptures at one end and the small coccoid bodies, which I designate spores, issue into the blood stream (*cf.* Fig. 5, page 489).

In my opinion, this multiple transverse fission is scarcely an essential phase of the Spirochaete in the Vertebrate host, but may occur at the crisis, and may explain the 'after phase.' I regard the phenomenon largely as an anticipation of what occurs in the Invertebrate host, which is frequently a tick. Such resistant, sporular 'granules' may occur in or on the mammalian red cells in the case of *S. duttoni* or *S. recurrentis*, and may be seen sometimes apparently inside the avian red blood corpuscles in the case of *S. marchouxi*. Empty periplastic sheaths, from which the 'granules' have issued may sometimes be seen lying in the neighbourhood.

Balfour (1908) has stated that small ovoid bodies, or granules, formed by *Spirochaeta marchouxi* occur within the blood corpuscles. I have seen similar bodies on a few occasions. Prowazek (1906) recorded intra-corpuscular stages of *S. marchouxi*, and Breinl (1907) observed *S. duttoni* forming granules in the spleen.

I have also observed in some smears of human blood from a case which had apparently recovered from African tick fever, some

interesting intra-corpuscular forms of *S. duttoni*. The Spirochaetes appeared as spiral bodies with terminal swellings somewhat resembling spermatozoa. These Spirochaetes are like forms of *S. nicollei* (a variety of *S. marchouxi*) figured by Blanc (1911)* as occurring in the haemocoelic fluid of *Argas persicus*.

SOME OBSERVATIONS ON SPIROCHAETES (*S. DUTTONI, S. MARCHOUXI*) IN TICKS (*ORNITHODORUS MOUBATA*,† *ARGAS PERSICUS*†)

The foregoing is a brief account of the main results of my investigations of certain blood Spirochaetes in the Vertebrate host. With regard to stages of some of these organisms in the Invertebrate there is less definite information. Dutton and Todd, in 1905, showed that the tick *Ornithodorus moubata* was the carrier of *Spirochaeta duttoni*, and they saw the passage of the Spirochaete through the gut-wall into the body-cavity of the tick. They also demonstrated that hereditary infection of the ticks occurred, as did Koch. After an interval of some four years, Sir Wm. Leishman investigated further the exact method of transmission of *S. duttoni* by *O. moubata*. Since the early part of 1909 I have had the opportunity, both in Cambridge with Professor Nuttall, and in Liverpool, of carrying out some experiments confirming Leishman's work. Hindle (1911) has also recently confirmed the same. There is no need, then, for me to set forth my experiments in detail, but the results of laboratory experiments of mine may be briefly summarised as showing that infection of the salivary glands is not the common mode of infection (as was supposed by Koch), that the excretion from the Malpighian tubules of the tick is infective, and passes near the end of the period of feeding into the wound caused by the tick's bite; that within the adult tick the Spirochaetes undergo change, producing small forms. Some of the Spirochaetes in the intestine of the tick resist digestion therein to varying degrees. They may disappear as such in a few hours after the tick has fed; they usually disappear in a few (3 to 10) days, but may remain in the intestine, as

* It is to be regretted that in the earlier portions of Blanc's memoir (dealing with the structure, division and classification of Spirochaetes in general) the contents of several important memoirs are quite overlooked, although the papers in question are listed in his bibliography.

† The *Ornithodorus* came from Uganda, the *Argas* from Egypt.

Spirochaetes, for two or three weeks. This phenomenon partly depends on the temperature at which the tick is kept, 37° C. being an optimum for development of the Spirochaetes in the tick. Some Spirochaetes pass through the gut-wall of the tick and reach the haemocoelic cavity, where they may attach themselves to the colourless corpuscles floating in the haemocoelic fluid. The Spirochaetes then break up by multiple transverse fission into coccoid bodies (spores) composed of densely staining chromatin surrounded by a thin covering of cytoplasm, like those described in the blood. Certain Spirochaetes become intra-cellular in the gut-epithelium and alimentary diverticula, and may produce granules there. Ultimately some of the coccoid bodies reach the ovaries and ova, as well as the Malpighian tubules of the tick, where they may multiply within the cells of these organs.

STAGES IN THE TICK EMBRYO

As Leishman, Balfour, Hindle, and Blanc have recently published their observations in some detail it is quite unnecessary for me to recapitulate their work, consequently I will merely summarise my results.

It has been mentioned that Spirochaetes in the tick have the power of penetrating the gut-wall, reaching the body cavity and there in the haemocoelic fluid and its cellular elements forming minute ovoid or rod-like bodies. In the course of either the movement of the Spirochaetes or of the haemocoelic fluid, the ovoid bodies reach the ovary, where they intermingle with the developing ova, and become incorporated with some of them. The eggs when laid may contain these minute bodies. Recently laid eggs of *Ornithodorus* and *Argas*, crushed, made into an emulsion with a little sterile salt solution, and then inoculated into mice or chickens, were not often infective. On the other hand, when the eggs were kept in an incubator at 34° to 37° C. for four to six days before being injected, the experimental animals developed spirochaetosis and died in a short time (3 to 6 days). When bacillary forms had developed after keeping the eggs at 24° C., the contents of the crushed eggs*

* In experiments with eggs, the contents of 6 to 12 eggs were used each time.

were infective in three experiments in four to seven days. The results of my microscopical examination of tick eggs are as follows:—

1. Egg when laid shows no Spirochaetes. Extremely thin smears show a few ovoid bodies which are difficult of detection.

2. Egg three to five days incubated. The embryonic Malpighian tubes are developed. Some of the yolk is absorbed. The ovoid bodies can be more easily detected as groups in the Malpighian tubules. A few have begun to elongate.

3. An egg six to seven days incubated shows more organs of the tick formed. Many of the ovoid bodies have lengthened and become bacillary. At this stage they may rupture the cell in which they developed, and escape into the lumen of the Malpighian tubule.

4. Owing to development of organs it is difficult to follow the metamorphosis of bacillary or vibrio forms into fully formed Spirochaetes, but two methods seem possible (*a*) fusion of rods; (*b*) elongation and growth in thickness of bacillary forms. Probably the latter method chiefly occurs.

5. A recently hatched infective tick contains in its gut (*a*) ovoid bodies; (*b*) bacillary forms; (*c*) a very few fully developed Spirochaetes if kept at 35° C. for six or eight days.

STAGES IN TICK NYMPHS BORN OF INFECTED PARENTS

Nymphs of *Argas persicus* or of *Ornithodorus moubata*, born of infected parents, usually contain coccoid bodies (spores) and bacillary forms (which are elongating spores).

Experiments with such nymphs of *O. moubata*, kept at 24° C., show that they are capable of infecting mice with *S. duttoni* when fed on them. Three experiments positive.

Two experiments with two nymphs of *A. persicus*, born in the laboratory of infected parents, kept at 24° C. for six days, and fed on young pigeons, were negative. Similar results were obtained by Brumpt and by Blanc,* but my experiments are too few for generalisation as to the infectivity of nymphs of *Argas persicus*. Again, such nymphs may possess a natural immunity, or they may come from eggs which did not happen to become infected, or

* See Blanc (1911), p. 102. This author doubts the connection of 'granules' with the life-cycle of *S. nicollei* in *Argas*. See his 5th paragraph on p. 112.

possibly the nymphs required warming to a higher temperature (say 34° C.). I had no more nymphs for further experiments at the time.

SPIROCHAETES OF LAMELLIBRANCHS

Regarding the Spirochaetes of Lamellibranchs, I have already published several papers (1907, 1908, 1909) dealing with the morphology and division of these organisms, and my recent results merely confirm these. Naturally, morphological features can be elucidated in greater detail in the case of these larger organisms than in the case of the blood Spirochaetes. Membranes are easily seen in life and nuclear detail can be observed by the use of the paraboloid condenser, as has been shown by some previous workers (Porter, 1909). In this respect I must beg to differ from Gross (1910) in his view that Spirochaetes are enucleate.

With regard to division, both longitudinal and transverse division occur, and each is of the same type as seen in the blood Spirochaetes (*cf.* Figs. 2 and 3), and the same description applies. In transverse division the direct method without 'incurvation' appears to be common. The figures of 'incurvation' division published by Gross and others, are, in my opinion, far from convincing. Movements causing 'deception division' were described by Porter in 1909, and their significance stated. Recently, a revival of these rather old ideas has occurred, with the result that perfectly normal methods of longitudinal and transverse division have been overlooked, since attention was focussed on the rarer and possibly abnormal modes. I would direct attention to a paper by Porter (1909), in which a careful study was made of many peculiar movements of living Spirochaetes from Lamellibranchs. Also those Spirochaetes undergoing longitudinal division were again shown to be provided with two membranes, produced by the splitting of the original one, thus further confirming my work (1907-8-9). Borrel has also noted the occurrence of a double membrane in some specimens of *S. balbianii*. Further, double (that is, divided) chromatin granules can be seen, after staining, in Spirochaetes about to divide. Such have been figured by some authors who regard transverse division as the only mode (*cf.* Calkins, 1909, p. 221).

Spirochaeta balbianii of oysters, *Tapes* and other molluscs, *S. anodontae* and *S. solenis*, *n.sp.*, have been under my observation

for some years. During this time, small ovoid bodies ('spores') of the same diameter as the Spirochaetes have been found from time to time (*cf.* Fig. 5*e*) and also empty sheaths, in both the crystalline style, the intestinal contents and the water in which the Lamellibranchs were living. The significance of these forms was considered, but until the actual formation of them from Spirochaetes had been repeatedly seen (and confusion with extraneous bodies excluded), I did not wish to publish my results. Since cross-infection of the molluscs has been proved to occur by the Spirochaetes swimming out of the alimentary tract and mantle cavity of the mollusc into the surrounding water (Fantham, 1907-8), the production of spores has not so great a significance as in the case of the blood

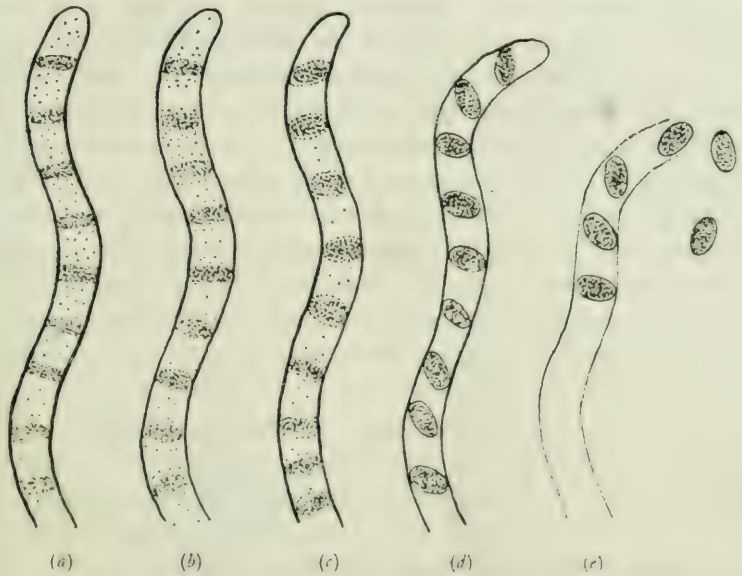


FIG. 5.—Shows formation of ovoid or coccoid bodies (as in *S. balbianii*) within part of Spirochaete. All details of membrane, etc., omitted for clearness' sake. (*a*) Normal form with chromatin bars. Tenuous cytoplasm. (*b*) Concentration of protoplasm round bars beginning. (*c*) Ovoid bodies differentiating. (*d*) Fully formed ovoid bodies within periplast. (*e*) Periplast ruptured and degenerating. Ovoid or coccoid bodies (spores) escaping.

forms. Bosanquet (1911) has also mentioned, in a recent note, the formation of coccoid bodies in a preparation containing *S. anodontae*. The method of formation of spores (Fig. 5 *a-e*) is identical with that seen in the blood Spirochaetes. I have seen spores issuing from *S. balbianii* and *S. anodontae* on several occasions (Fig. 5*e*).

The spores or coccoid bodies are probably able to withstand conditions unfavourable to the spirochaetiform stage of the parasite. Spores may also be formed in a fresh preparation of Spirochaetes kept moist for twenty-four hours. However, I do not consider that the coccoid bodies are products of degeneration, as degenerating Spirochaetes have a very different appearance.

Cross-infection by the agency of water has been shown. I have infected apparently clean *Tapes aureus* with *S. balbianii* by placing an infected oyster with them. Infected *Tapes* placed in water with a clean stock of *Tapes* result in all becoming infected. Similar experiments with *Ostrea edulis*, *Pecten jacobaeus** and *Tapes aureus* had the same result. *Sphaerium corneum* has been cross-infected with Spirochaetes from *Anodonta cygnea*, though with more difficulty. Water in the aquaria or basins in which infected individuals were placed has yielded Spirochaetes, and healthy molluscs introduced into this water have become infected. An intermediate host does not seem necessary for the transference of the Spirochaetes. Various commensals of oysters and anodons have been examined. *Atax bonzi*, from the mantle cavities of infected *Anodonta cygnea*, have been dissected, and in some of them, spores or bodies closely resembling them, have been found. Some of these bodies become rod-like; but as the complete metamorphosis of them into Spirochaetes has not been observed, it is well for the present, to consider them as under suspicion of being evolutionary stages of *S. anodontae*, though they might be separate bodies.

Previously, mention has been made of attempts to disprove longitudinal division by suggested 'explanations' that break down.

Much trouble has arisen in this and in other connections from a paper by Gross (1910) on the spirochaetal parasites of *Pecten*. Both Gross and those who have followed his lead, unfortunately show a regrettable lack of knowledge of the literature on the group, more particularly in connection with the subject of division. Had they noted carefully the paper by Miss Porter and myself (1909), and another dealing with movements simulating division by Porter (1909), it seems probable that they would not have assumed that transverse division following 'incurvation' had been mistaken for

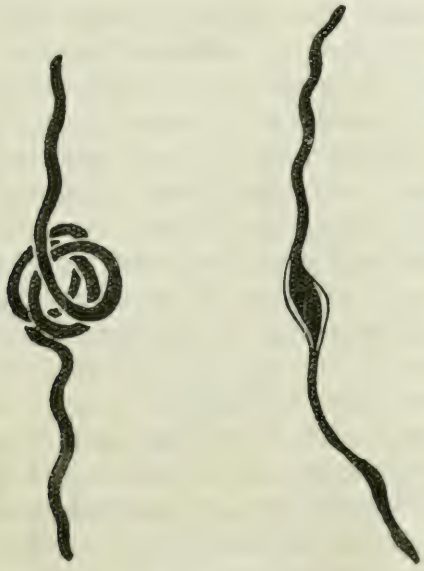
* In consequence of infection experiments, I consider that *Cristispira pectinis* (Gross) is really *Spirochaeta balbianii*.

longitudinal fission, nor that the mechanism of division could be confused when there are such striking differences in movements.

Some writers have stated that Spirochaetes are homogeneous and undifferentiated in structure. The instructive results of compression of these organisms should be noted, and then the undoubted differentiation into periplast and protoplasm no longer presents difficulties. I have performed such experiments myself, and by crushing *S. balbianii*, the bars of chromatin issue intact with the cytoplasm and take the characteristic coloration on staining. The mode of formation of spores is a further indication of internal differentiation.

Encystment of Spirochaetes has been described by several writers. Up to the present, neither in the Spirochaetes of the blood nor in those of Lamellibranchs have I found true encystment. Two types of pseudo-cyst forms, have, however, been encountered:—

1. The Spirochaete becomes more closely coiled, either about its centre or nearer one end, so that a ball-like form is produced. This ball simulates a cyst with the body of the Spirochaete protruding



(*a*) (*b*)

Fig. 6.—Pseudo-cysts. (*a*) Ball-like coil in centre of Spirochaete, which soon uncoils. (*b*) Plasmatic cyst or swelling.

from either end (Fig. 6*a*). But this form is only temporary, the Spirochaete uncoiling after a short time and swimming away normally.

2. Plasmatic cysts may be formed. Here the Spirochaete is dying, and so is not normal. The periplast relaxes and the cytoplasm tends to collect into small irregular masses or droplets, which cause local bulgings along the body. I have not seen these protoplasmic aggregations other than in animals in an almost moribund condition, when the Spirochaetes, naturally, were under unfavourable conditions (Fig. 6*b*). One or two similar plasmatic cysts may occasionally be found under similar conditions on a blood Spirochaete.

NOMENCLATURE

The re-naming of the Spirochaetes of Lamellibranchs by Gross (1910) as *Cristispira*, is, in my opinion, a mistake, is quite unnecessary, and should be disregarded. The Spirochaete group as a whole, (or various members of it), has received so many names that it seems to be a mania to re-name it according to individual fancy, as witness *Borrelia*, *Spiroschaudinnia*, *Spiroflagellata*, *Proflagellata*, *Spironemacea*, and now *Cristispira!*

The accounts of *Spirochaeta plicatilis*, the type species of the genus, given by Ehrenberg, by Schaudinn and by Zuelzer (1910) vary so much that they cannot be reconciled, and suggest that the authors may have dealt with different organisms. Zuelzer's work certainly needs confirmation. Hence, conclusions involving the structure of *S. plicatilis* cannot be accepted as a basis for changes in the nomenclature of the group. It should also be remembered that *S. plicatilis* is said to undergo multiple transverse fission, and in this respect resembles the Spirochaetes of Lamellibranchs and of the blood. It seems, then, that the new generic name *Cristispira* is unnecessary. It is much to be deplored that there are so many attempts at fresh classifications and nomenclature before the life-histories of the organisms are completely known.

The introduction of the term 'crista' for the membrane also is unnecessary. In 1907-8, I pointed out that there was a difference between the membrane of a Spirochaete and the undulating membrane of a Trypanosome, for in Spirochaetes the membrane

does not markedly undulate. (Fantham, 1908, pp. 31, 55, 58.) Hence, I referred constantly to the structure in Spirochaetes as a membrane only.

Quibbles as to whether bars or rings of granules of chromatin are present in the body of a Spirochaete, also speculations as to the exact shape of the organism need not have arisen, for the matter was discussed fully by me in 1908, and illustrated (text-fig. 6, p. 30) by drawings of sections of Spirochaetes cut *in situ* in the style of Anodon. I have since (1909) cut sections of infected styles of *Tapes aureus*, and have figured (Pl. VI, fig. 54) the sections of the Spirochaetes therein. Careful examination of these figures would be sufficient to settle such questions as those of shape of the body and disposition of the membrane to the satisfaction of any thinking person.

An interesting observation by Balfour (1911) may be noted here. He states that '*Treponema pallidum* is a granule shedder.' In that case, it may be that *T. pallidum* is really a member of the genus *Spirochaeta*, too minute for observation of a membrane or internal chromatin granules, and so its coils may only *appear* to be fixed. Further, the 'granules' of *Trep. pallidum* may explain the *Cytoryctes luis* of Siegel.

The numerous similarities between blood Spirochaetes and those of Lamellibranchs, then, justify the retention of them in the same genus. The morphology of their bodies, membranes and nuclei is similar. They divide by both longitudinal and transverse division. At one stage in their life-history they produce small, oval bodies within their periplasts. When liberated, these oval bodies serve either for re-infection of the same host or for cross-infection purposes. Hence, the life-history is on parallel lines.

SUMMARY

1. The Spirochaetes considered in this paper are *S. duttoni*, *S. recurrentis* and *S. marchouxi* (= *gallinarum*) among blood-inhabiting forms, also *S. balbianii* in *Ostrea edulis* and *Tapes aureus*, *S. anodontae* in *Anodonta cygnea* and *S. solenis** in *Solen ensis*. Both living and stained material have been used.

* *S. solenis* is about 40μ to 60μ in length in the specimens which I have measured. It is the salt-water counterpart of *S. anodontae*, both having pointed ends.

2. True longitudinal division, as well as transverse division has been observed in these Spirochaetes. There is a periodicity in the division of the blood-inhabiting Spirochaetes, transverse division occurring when the parasites are numerous in the blood, longitudinal division occurring at the beginning and end of infection.

3. Transverse division following flexion, or 'incurvation,' has been observed, but somewhat rarely. Transverse division usually occurs in relatively straight or unflexed forms. I do not consider that 'incurvation' is a necessary preliminary of transverse division.

Intertwined forms have not been mistaken for longitudinal division.

4. The protoplasmic contents of some of the Spirochaetes of the blood may break up into a number of small, round, or ovoid bodies, lying loose within the periplast, which ultimately ruptures at one end and sets them free. These minute bodies, variously known as 'coccoid bodies,' 'granules,' or 'spores,' are formed at the crisis. I doubt if these bodies represent an essential phase in the life-history of the Spirochaetes in the Vertebrate host, but are rather an anticipation of the similar phase in the Invertebrate hosts of these Spirochaetes. However, occasionally 'granules' may occur inside the red-blood cells.

5. Certain *S. duttoni*, when ingested by *Ornithodorus moubata*, and certain *S. gallinarum* ingested by *Argas persicus* pass through the intestinal wall of their hosts, and then form minute coccoid bodies, spores, or 'granules' by multiple transverse fission. Such granules, as well as Spirochaetes, may be found in the haemocoelic fluid of the ticks, in the Malpighian tubules and in the gonads.

6. Some of the Spirochaetes and spores reach the ovaries and ova of the infected parent tick. The spores concentrate in the Malpighian tubules of the developing embryo, which may be born infected.

7. Many nymphs of *O. moubata* born of infected parents are themselves capable of infecting. In the case of nymphs of *Argas persicus*, although various observers have recorded negative results, more experiments are necessary before it can be asserted that nymphs born of infected parents are themselves not infective.

8. The main source of infection from both adult and young ticks is the white excrement passed from the Malpighian tubules.

9. Elongation of the coccoid bodies, spores or 'granules' to

form short rods, and growth of these rods to form longer (or vibrio) forms has been observed in the tick. In this way young Spirochaetes are developed.

10. The Spirochaetes of Lamellibranchs do not necessarily depend on a carrier for change of Lamellibranch host. Cross-infection is brought about by water, which conveys not only active living Spirochaetes from the alimentary tract and mantle cavity of infected molluscs to the inhalent apertures of other molluscs, but also coccoid bodies (spores) may be thus conveyed and cross-infect. Coccoid bodies have been observed in process of formation in *S. balbianii* and *S. anodontae*. (Fig. 5.)

11. The life-cycle of the Spirochaetes of Lamellibranchs and of the Spirochaetes of the blood of Vertebrates follows a similar course. Their morphology is much the same, allowing for differences of size. There appears to be no justification for separating generically the Spirochaetes of Lamellibranchs from their allies in the blood of Vertebrates. (See p. 492.)

ADDENDUM

From a recent communication I gather that Frl. Dr. M. Zuelzer has in the press another paper on *Spirochaeta plicatilis*. Unfortunately, at the time of correcting proofs of this paper, Frl. Zuelzer's memoir is not published, and I am unaware of her conclusions. However, I should like to state, with all due respect to the various authors who have written on Spirochaetes, that it seems to me that much of the recent work on the group has been done by inexperienced investigators who, in consequence of their inexperience, are prone to make dogmatic and contradictory statements based on slender evidence, and have a penchant for putting forward new classifications. The literature on Spirochaetes at present is, in consequence, in a state of the utmost confusion. As one who has worked on many Spriochaetes in various parts of the world since 1906, I wish to state emphatically that I do not think serious attention should be paid to any work which does not set forth in clear and concise language the practically complete life-cycle of the organism under discussion, with clear and convincing reasons for any suggested new classification and appropriate illustrations of new features.

REFERENCES

This list is, of necessity, incomplete. Further references will be found at the ends of some of the papers cited.

Balfour, A. (1908). 'Spirochaetosis of Sudanese Fowls.' 3rd Report of Wellcome Labs., Khartoum, pp. 38-58.

Balfour, A. (1911*a*). 'The rôle of the Infective Granule in certain Protozoal Diseases.' Brit. Med. Journ., No. 2654, pp. 1268-1269.

Balfour, A. (1911*b*). 'Spirochaetosis of Sudanese Fowls.' 4th Report of Wellcome Trop. Labs., Khartoum, Vol. A Medical, pp. 76-107.

Blanc, G. R. (1911). 'Les Spirochètes: Leur évolution chez les Ixodidae.' 129 pp., 2 plates. Paris, Jouve et Cie.

Bosanquet, W. C. (1911). 'Brief notes on the Structure and Development of *Spirochaeta anodontae*.' Quart. Journ. Microsc. Sci., Vol. LVI, pp. 387-393, 1 plate.

Breinl, A. and Kinghorn, A. (1906). 'An Experimental Study of the Parasite of the African Tick Fever.' Liverpool School of Trop. Med., Mem. XXI.

Breinl, A. (1907). 'The Morphology and Life History of *Spirochaeta duttoni*.' Ann. Trop. Med. and Parasitol., Vol. I, pp. 435-438, 1 plate.

Brumpt, E. (1909). 'Existence d'une Spirochétose des Poules à *S. gallinarum* dans le pays Somali.' C.R. Soc. Biol., t. LXVII, pp. 174-176.

Calkins, G. N. (1909). 'Protozoölogy,' 349 pp. Lea & Febiger, New York and Philadelphia. ['The genus *Spirochaeta* and allies,' pp. 217-232. (A good account of the Spirochaete group)]

Dutton, J. E., and Todd, J. L. (1905). 'The Nature of Human Tick Fever in the Eastern Part of the Congo Free State.' Liverpool Sch. Trop. Med., Memoir XVII.

Ehrenberg, C. G. (1833-35). '*Spirochaeta*, n.g., *Spirochaeta plicatilis*, n.sp.,' Abh. Akad. Berlin, p. 313.

Fantham, H. B. (1907). '*Spirochaeta* (*Trypanosoma*) *balbianii*, its movements, structure, and affinities; and on the Occurrence of *Spirochaeta anodontae* in the British Mussel, *Anodonta cygnea*.' (Prelim. Communic.) Ann. & Mag. Nat. Hist., ser 7, Vol. XIX, pp. 493-501.

Fantham, H. B. (1908). '*Spirochaeta* (*Trypanosoma*) *balbianii* (Certes) and *Spirochaeta anodontae* (Keysselitz): Their movements, structure, and affinities.' Quart. Journ. Microsc. Sci., Vol. LII, pp. 1-73, 3 plates.

Fantham, H. B. (1909). 'The Spirochaetes found in the Crystalline Style of *Tapes aureus*: A Study in Morphological variation.' Parasitology, Vol. II, pp. 392-408, 1 plate.

Fantham, H. B., and Porter, A. (1909). 'The Modes of Division of *Spirochaeta recurrentis* and *S. duttoni* as observed in the Living Organisms.' Proc. Roy. Soc., B, Vol. LXXXI, pp. 500-505.

Gross, J. (1910). '*Cristispira*, nov. gen. Ein Beitrag zur Spirochätenfrage.' Mittheil. zool. Stat. Neapel., Vol. XX, pp. 41-93, 1 plate.

Hindle, E. (1911). 'The Transmission of *Spirochaeta duttoni*.' Parasitology, Vol. IV, pp. 133-149.

Leishman, W. B. (1909). 'Preliminary Note on Experiments in connection with the Transmission of Tick Fever.' Journ. Roy. Army Med. Corps, Vol. XII, pp. 123-135.

Leishman, W. B. (1910). 'An Address on the Mechanism of Infection in "Tick Fever" and on Hereditary Transmission of *Spirochaeta duttoni* in the Tick.' Lancet, Vol. CLXXVIII, pp. 11-14. Also Trans. Soc. Trop. Med. & Hyg., III, pp. 77-95.

Nuttall, G. H. F. (1904). 'Ticks and Tick-transmitted Diseases.' Trans. Epidemiol. Soc. Lond., Vol. XXIV, pp. 12-26. (*S. marchouxi*, see table p. 16).

Porter, Annie (1909). 'Some Observations on Living Spirochaetes from Lamellibranchs.' Arch. Zool. Expér. et Gén., 5e Ser., T. III, pp. 1-26.

Prowazek, S. (1906). 'Morphologische und Entwicklungsgeschichtliche Untersuchungen über Hühnerspirochaeten.' Arb. a. d. Kaiserl. Gesundheitsamte, Vol. XXIII, pp. 554-568. 2 plates.

Stephens, J. W. W. (1906). 'A Note on the Structure of *Spirochaeta duttoni*.' Lancet, No. 4329, (Aug. 18), p. 438.

Stephens, J. W. W., and Christophers, S. R. (1904). 'Prac. Study of Malaria, etc.' 2nd edition. London, Williams & Norgate (*S. gallinarum*, p. 378).

Swellengrebel, N. H. (1907). 'Sur la cytologie comparée des Spirochètes et des Spirilles.' Ann. Inst. Pasteur, Vol. XXI, pp. 448-465 and 562-586, 2 plates.

Zuelzer, M. (1910). 'Über *Spirochaeta plicatilis* und *Spirulina*.' Zool. Anzeiger, Vol. XXXV, pp. 795-797.

DESMOGONIUS DESMOGONIUS, A NEW SPECIES AND GENUS OF MONOSTOME FLUKES

BY

J. W. W. STEPHENS, M.D., CANTAB.

(*Received for publication* 20 *November*, 1911)

Some half-dozen flukes were found by Prof. R. Newstead in the alimentary canal of an edible Nicaraguan turtle (*Chelone mydas*), that died on board ship off Jamaica. They formed part of the collection of the 23rd Expedition of the Liverpool School of Tropical Medicine.

The colour in life was blood red. They were placed in salt solution, as no fixative was available. On coming into my possession they were gradually transferred to glycerine. The following description is based on the examination of a single specimen, as the rest of the material was lost. Though incomplete, yet I think it is sufficient for establishing a new genus.

The body is concavo-convex, 5·2 mm. long by 1·8 mm. The skin has no scales. It is pointed anteriorly and rounded posteriorly, where it is furnished with two conical protuberances. The head possesses no collar.

The alimentary tract.—Oral sucker is spherical, and has a diameter of 0·45 mm. This is followed by a short oesophagus. The gut caeca run almost to the extreme posterior end on either side. They are characterized by numerous short lateral branches internally and externally. The arrangement appears to be essentially the same as that in the genus *Charaxicephalus*.

The common genital pore lies on the left side of the body about 2 mm. from the anterior extremity, just in front of the uterine

coils and external to the left caecum. Cirrus pouch present, lying between the gut caeca, about a millimetre behind the posterior border of the sucker, and followed by a curved seminal vesicle.

The testes are split up into a number of spherical parts. These on each side form a chain, not only external to the uterine coils, but also to the gut caeca, and almost completely anterior to the vitellaria with the exception of the last one or two. The right testis is divided into eight parts and the left into seven parts.

The ovary.—Slightly to the right of the mid-line, and slightly behind the level of the posterior vitellarian acini is apparently globular, but no details were recognisable.

The vitellaria, situated in the posterior third of the body, lie outside the uterine coils overlapping the gut caeca and commence slightly anterior to and internal to the last division of the testes. The follicles are not split up to the extent they are in *Charaxicephalus*. They consist of a number of follicles to some extent arranged in groups. The transverse vitelline duct runs from the posterior extremity of the vitellaria on each side obliquely towards the middle line into a vitelline receptacle.

Eggs.—Operculate, 33 × 15 μ, with a tuft of long filaments at each pole.

Looss (1902) in his key for determining the genera of the Pronocephalidae gives the following classification:—

1. Mit 2 seitlich der Mitellinie gelegenen einfachen Hoden, Keimstock vor ihnen (2).

Mit 2 ebenfalls seitlich gelegenen Hoden, deren jeder in eine Anzahl hinter einander gelegener Theilstücke zerfallen ist; Keimstock hinter ihnen; Darmschenkel sowohl wie der Schenckel der Excretionsblase mit Seitenzweigen; Körper hinten in 2 stumpf conische Fortsätze auslaufend .....*Charaxicephalus*.

The present genus closely resembles *Charaxicephalus* (1) in the fact that the testes are split up into a number of parts, and (2) that the ovary lies behind them. (3) In the presence of two bluntly conical appendages posteriorly. (4) The gut caeca reach the posterior extremity and are provided with lateral appendages internally. (5) The eggs are provided with a tuft of filaments.

It differs from it, however, in the following points:-

1. There is no collar.
2. The testes are situated external to the gut caeca and form a chain on each side.
3. The common genital pore opens external to the gut caecum.
4. The vitellaria occupy the posterior third (not posterior half).

I propose,* therefore, to make for this fluke a new genus, for which I suggest the name *Desmogonius*, and for the specific name also *desmogonius*.

LITERATURE

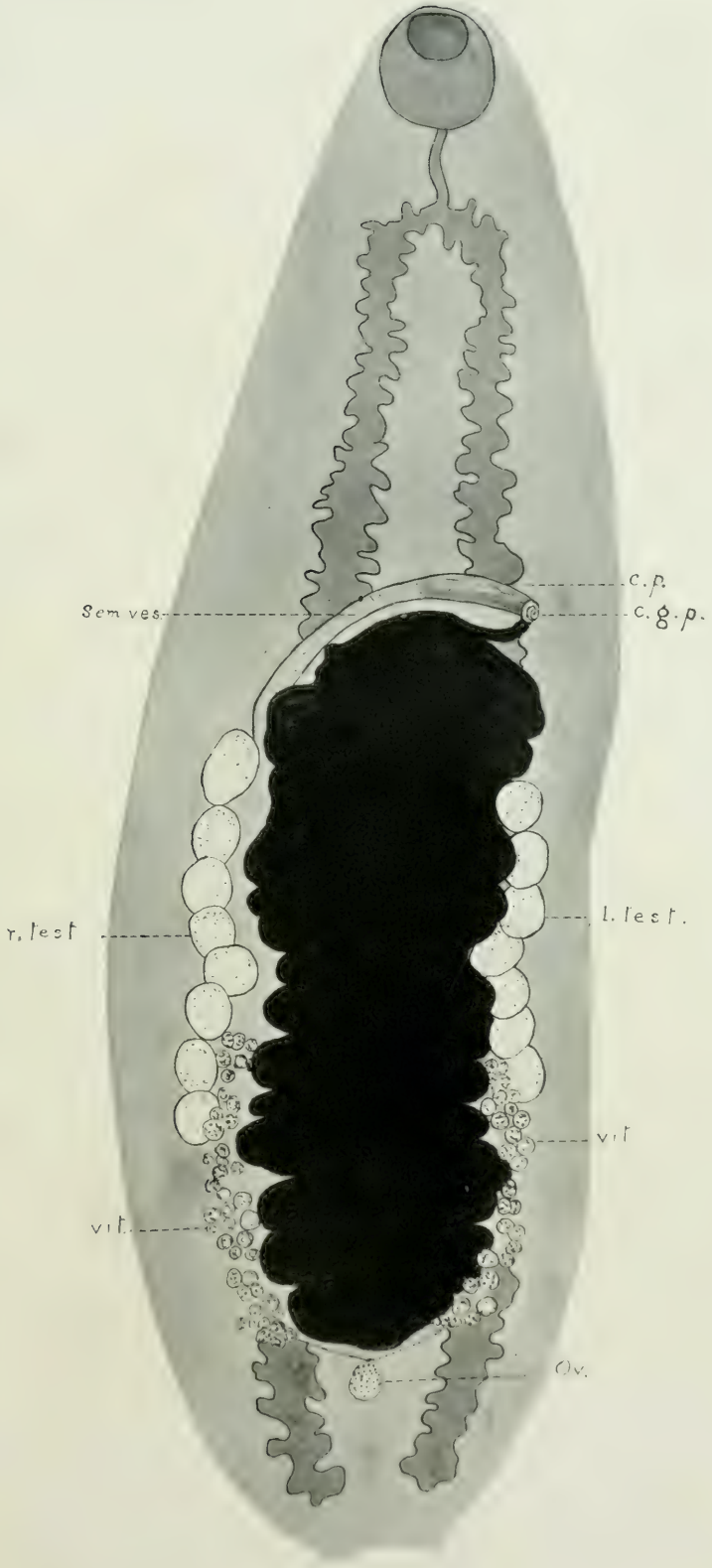
Looss, A. (1902) Ueber neue und bekannte Trematoden aus Seeschildkröten. Zool. Jahrb., Abth. f. Syst., Bd. XVI.

* Professor Looss, who kindly examined the fluke for me, informed me that it must be separated from other genera of the Pronocephalidae.

PLATE XXIII

Desmogonius desmogonius × 40.

sem. ves. = seminal vesicle.
c. p. = cirrus pouch.
c. g. p. = common genital pore.
r. testis = right testis.
l. testis = left testis.
vit. = vitelline glands.
ov. = ovary.


c.p.
Sem ves.
c.g.p.
r. test
l. test.
vit.
vit.
Ov.

Volume V February, 1912 No. 4

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY
THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Editor
PROFESSOR SIR RONALD ROSS, MAJOR I.M.S. (RET.), D.P.H., F.R.C.S., D.Sc., LL.D., F.R.S., K.C.B.

In Collaboration with
J. W. W. STEPHENS, M.D., CANTAB., D.P.H.
PROFESSOR R. NEWSTEAD, M.Sc., A.L.S., F.E.S., Hon. F.R.H.S.
PROFESSOR J. L. TODD, B.A., M.D., C.M. McGill, D.Sc., Liv
H. WOLFERSTAN THOMAS, M.D., C.M. McGill.
ANTON BREINL, M.U.DR.

C. Tinling & Co., Ltd.
Printers to the University Press of Liverpool
53 Victoria Street

THE INCORPORATED
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

(Affiliated with the University of Liverpool)

Hon. President: Her Royal Highness Princess Christian

Hon. Vice-Presidents: The Duke of Northumberland, K.G.
The Earl of Derby, G.C.V.O.
Earl Cromer, G.C.B.
Viscount Milner, G.C.B.
Lord Pirrie, K.P.
Sir Owen Phillips, K.C.M.G.
Mr. O. Harrison Williams

COMMITTEE

Chairman: Sir William H. Lever, Bart.

Vice-Chairman: Mr. F. C. Danson

Sir Alfred W. Dale	*University of Liverpool*
Sir W. B. Bowring, Bart.	*Council of University of Liverpool*
Professor Caton	
Professor Sherrington	*Senate of University of Liverpool*
Professor Herdman	
Dr. C. J. Macalister	*Royal Southern Hospital*
Mr. George P. Newbolt	
Mr. C. Booth (Jun.)	*Steamship Owners' Association*
Mr. T. F. Harrison	
Mr. A. R. Marshall	*Shipowners' Association*
Mr. W. Roberts	
Mr. Charles Livingston	
Mr. George Brocklehurst	
Mr. J. W. W. Danson	
Mr. J. A. Tinne	
Mr. Robert Rankin	
Professor E. W. Hope	
Mr. J. W. Alsop	
Mr. Alec L. Rea, *Hon. Treasurer*	

Mr. A. H. Milne, B.A., C.M.G., *Secretary.*

B 10, Exchange Buildings, Liverpool

1. At the University of Liverpool

Professors - - MAJOR SIR RONALD ROSS, K.C.B., F.R.C.S., D.P.H., M.D., D.Sc., LL.D., F.R.S., Nobel Laureate 1902. (Indian Medical Service, retired). *Sir Alfred Jones Professor of Tropical Medicine*
ROBERT NEWSTEAD, M.Sc., A.L.S., F.E.S., *Dutton Memorial Professor of Medical Entomology*

Lecturer - - JOHN WILLIAM WATSON STEPHENS, M.D., Cantab., D.P.H. *Walter Myers Lecturer*

Assistant Lecturers - HAROLD BENJAMIN FANTHAM, D.Sc., B.A. *Assistant Lecturer in Parasitology*
HAROLD WOLFERSTAN THOMAS, M.D., C.M.
R. STENHOUSE WILLIAMS, M.B., D.P.H., *Assistant Lecturer in Public Health Bacteriology*

Honorary Lecturers - J. O. WAKELIN BARRATT, M.D., D.Sc., (Lond.)
MAJOR JOSEPH FITZGERALD BLOOD, M.D., M.Ch. (Indian Medical Service, retired)
ERNEST EDWARD GLYNN, M.A., M.D., (Cantab.), M.R.C.P., M.R.C.S.
PROF. E. W. HOPE, M.D., D.Sc.
WILLIAM THOMAS PROUT, M.B., C.M.G.

Honorary Statistician WALTER STOTT

Assistant Entomologist HENRY FRANCIS CARTER, S.E.A.C. DIPL.

Bibliographer - - WALTER DRAWZ

Clerk to the Laboratory - NORA MURPHY

2. At the Runcorn Research Laboratories

Director - - - WARRINGTON YORKE, M.D.

Assistant - - - B. BLACKLOCK, M.D., D.PH.

3. At the Royal Southern Hospital, Liverpool

Physicians - - CHARLES JOHN MACALISTER, M.D., F.R.C.P.
JOHN LLOYD ROBERTS, M.D., M.R.C.P.
MAJOR SIR RONALD ROSS, K.C.B., F.R.S., etc.

Surgeons - - - D. DOUGLAS CRAWFORD, F.R.C.S.
ROBERT JONES, F.R.C.S.
GEORGE PALMERSTON NEWBOLT, M.B., F.R.C.S.

Tropical Pathologist - JOHN WILLIAM WATSON STEPHENS, M.D., Cantab.

Clinical Pathological Assistant - DAVID THOMSON, M.B., Ch.B., D.P.H.

4. On Expedition

HAROLD WOLFERSTAN THOMAS, M.D., C.M.
WARRINGTON YORKE, M.D.

Laboratory
JOHNSTON TROPICAL LABORATORY, LIVERPOOL UNIVERSITY

Research Laboratory
CROFTON LODGE, RUNCORN

Hospital
ROYAL SOUTHERN HOSPITAL, LIVERPOOL

Secretary's Office
B 10, EXCHANGE BUILDINGS, LIVERPOOL

NOTICE

The following courses of instruction will be given by the Liverpool School of Tropical Medicine during 1912:—

Full Course begins 6 January. Short Course begins 1 June.
Diploma Examination, 1 April. Certificate Examination, 29 June.
Full Course begins 15 September.
Diploma Examination, 16 December.

The full Course of Instruction is open to all qualified medical men, and the Examination to all students who have taken out this full course.

Fee for the full Course of Instruction—Thirteen Guineas.

Fee for the Diploma Examination—Five Guineas.

Fee for the Short Course of Instruction—Four Guineas.

Fee for the use of a School microscope during one term—Ten shillings and sixpence.

For prospectus and further information, application should be made to the Dean of the Medical Faculty, University of Liverpool.

The following have obtained the Diploma in Tropical Medicine of the University of Liverpool:—

Diploma in Tropical Medicine

Date of Diploma	
1904	Augustine, Henry Joshua
1904	Bennett, Arthur King
1904	Bruce, William James
1904	Byrne, John Scott
1904	Clayton, Thomas Morrison
1904	Dalziel, John McEwen
1904	Dee, Peter
1904	Greenidge, Oliver Campbell
1904	Hehir, Patrick
1904	Khan, Saiduzzafor
1904	Laurie, Robert
1904	Maclurkin, Alfred Robert
1904	McConnell, Robert Ernest
1904	Nicholson, James Edward
1904	Philipson, Nicholas
1904	Sharman, Eric Harding
1904	Thomson, Frank Wyville
1904	Walker, George Francis Clegg
1905	Anderson, Catherine Elmslie
1905	Brown, Alexander
1905	Caldwell, Thomas Cathcart
1905	Critien, Attilio
1905	Hooton, Alfred
1905	Hudson, Charles Tilson
1905	Illington, Edmund Moritz
1905	Macfarlane, Robert Maxwell
1905	Maddock, Edward Cecil Gordon
1905	Moore, James Jackson
1905	Nightingale, Samuel Shore
1905	Radcliffe, Percy Alexander Hurst
1905	Young, John Cameron
1906	Adie, Joseph Rosamond
1906	Arnold, Frank Arthur
1906	Bate, John Brabant
1906	Bennetts, Harold Graves
1906	Carter, Robert Markham
1906	Chisholm, James Alexander
1906	Clements, Robert William
1906	Dundas, James
1906	Faichnie, Norman
1906	Jeffreys, Herbert Castelman
1906	Mackenzie, Donald Francis
1906	Pailthorpe, Mary Elizabeth
1906	Palmer, Harold Thornbury
1906	Pearse, Albert
1906	Sampey, Alexander William
1906	Smithson, Arthur Ernest
1906	Taylor, Joseph van Someron
1906	Taylor, William Irwin
1906	Tynan, Edward Joseph

Date of Diploma	
1906	Watson, Cecil Francis
1906	Willcocks, Roger Durant
1906	Williamson, George Alexander
1907	Allan, Alexander Smith
1907	Allwood, James Aldred
1907	Bond, Ashton
1907	Branch, Stanley
1907	Collinson, Walter Julius
1907	Davey, John Bernard
1907	Donaldson, Anson Scott
1907	Fell, Matthew Henry Gregson
1907	Gann, Thomas William Francis
1907	Graham, James Drummond
1907	Hiscock, Robert Carroll
1907	Keane, Joseph Gerald
1907	Kennan, Richard Henry
1907	Kenrick, William Hamilton
1907	Le Fanu, George Ernest Hugh
1907	Mackey, Charles
1907	Maddox, Ralph Henry
1907	McCarthy, John McDonald
1907	Raikes, Cuthbert Taunton
1907	Ryan, Joseph Charles
1907	Vallance, Hugh
1908	Caverhill, Austin Mack
1908	Crawford, Gilbert Stewart
1908	Dalal, Kaikhusroo Rustomji
1908	Dansey-Browning, George
1908	Davidson, James
1908	Dickson, John Rhodes
1908	Dowdall, Arthur Melville
1908	Glover, Henry Joseph
1908	Greaves, Francis Wood
1908	Goodbody, Cecil Maurice
1908	Harrison, James Herbert Hugh
1908	Joshi, Lemuel Lucas
1908	Le Fanu, Cecil Vivian
1908	Luethgen, Carl Wilhelm Ludwig
1908	Mama, Jamshed Byramji
1908	McCay, Frederick William
1908	McLellan, Samuel Wilson
1908	Pearce, Charles Ross
1908	Schoorel, Alexander Frederik
1908	Smith, John Macgregor
1908	Stewart, George Edward
1908	Tate, Gerald William
1908	Whyte, Robert
1909	Abercrombie, Rudolph George
1909	Allin, John Richard Percy
1909	Armstrong, Edward Randolph
1909	Barrow, Harold Percy Waller
1909	Beatty, Guy
1909	Carr-White, Percy
1909	Chevallier, Claude Lionel
1909	Clark, William Scott
1909	Cope, Ricardo
1909	Fleming, William
1909	Hanschell, Hother McCormick
1909	Hayward, William Davey
1909	Henry, Sydney Alexander
1909	Innes, Francis Alexander

Date of Diploma	
1909	Jackson, Arthur Frame
1909	Kaka, Sorabji Manekji
1909	McCabe-Dallas, Alfred Alexander Donald
1909	Meldrum, William Percy
1909	Murphy, John Cullinan
1909	Samuel, Mysore Gnananandaraju
1909	Shroff, Kawasjee Byramjee
1909	Thornely, Michael Harris
1909	Turkhud, Violet Ackroyd
1909	Webb, William Spinks
1909	Yen, Fu-Chun
1910	Brabazon, Edward
1910	Castellino, Louis
1910	Caulcrick, James Akilade
1910	Dowden, Richard
1910	Haigh, William Edwin
1910	Hamilton, Henry Fleming
1910	Hefferman, William St. Michael
1910	Hipwell, Abraham
1910	Homer, Jonathan
1910	Houston, William Mitchell
1910	James, William Robert Wallace
1910	Johnstone, David Patrick
1910	Korke, Vishnu Tatyaji
1910	Macdonald, Angus Graham
1910	Macfie, John Wm. Scott
1910	Manuk, Mack Walter
1910	Murison, Cecil Charles
1910	Nanavati, Kishavlal Balabhai
1910	Nauss, Ralph Welty
1910	Oakley, Philip Douglas
1910	Pratt, Ishmael Charles
1910	Sabastian, Thiruchelvam
1910	Shaw, Hugh Thomas
1910	Sieger, Edward Louis
1910	Sousa, Pascal John de
1910	Souza, Antonio Bernardo de
1910	Waterhouse, John Howard
1910	White, Maurice Forbes
1911	Blacklock, Breadalbane
1911	Brown, Frederick Forrest
1911	Chand, Diwan Jai
1911	Holmes, John Morgan
1911	Ievers, Charles Langley
1911	Iles, Charles Cochrane
1911	Ingram, Alexander
1911	Kirkwood, Thomas
1911	Knowles, Benjamin
1911	Liddle, George Marcus Berkeley
1911	Lomas, Emanuel Kenworthy
1911	Mackarell, William Wright
1911	MacKnight, Dundas Simpson
1911	Mascarenhas, Joseph Victor
1911	Murray, Ronald Roderick
1911	Oluwole, Akidiya Ladapo
1911	Rao, Koka Ahobala
1911	Sinton, John Alexander
1911	Tarapurvalla, Byramji Shavakshah
1911	Taylor, John Archibald
1911	Woods, William Medlicott

EDITORIAL NOTICE

By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the fourth number of the fifth volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine or Hygiene may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

NOTES ON SOME BLOOD-PARASITES IN MAN AND MAMMALS

BY

HARALD SEIDELIN.

(*Received for publication* 15 *November*, 1911)

I.

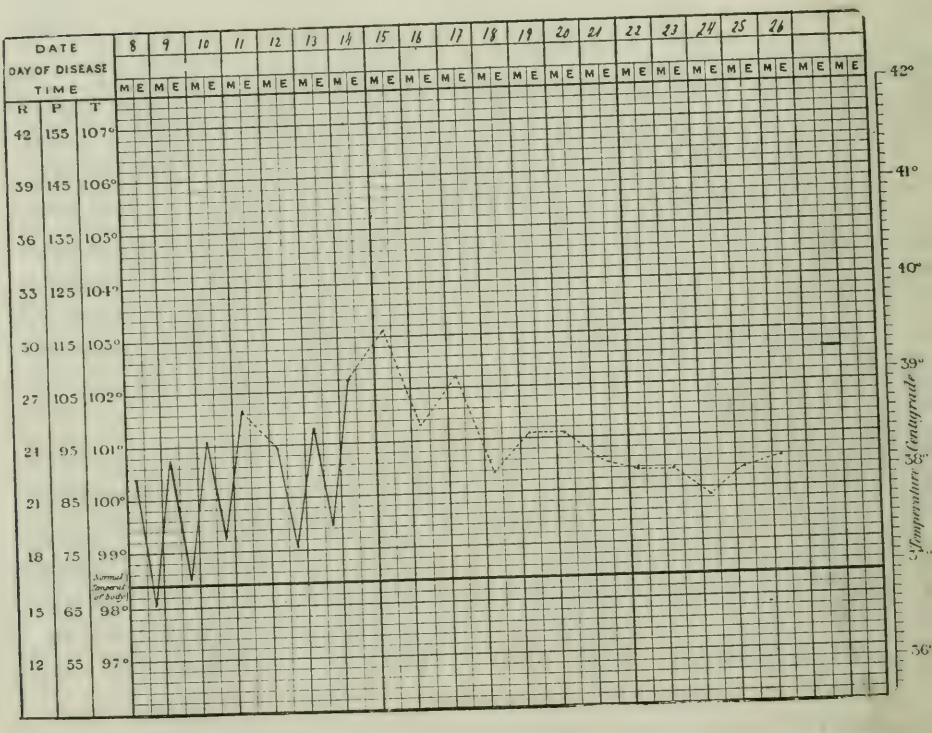
In a previous paper (1910) I briefly mentioned that I had observed, in Yucatán, various cases of non-classified fevers and that in one case I had found, in the blood, elements of an apparently parasitic nature. The publication of further particulars has been delayed in the hope of receiving material from similar cases, but it now seems advisable, although such material has not been obtained so far, to publish a summary of the one case observed, since it presents some special interest, when considered in connection with my observations (1909, 1911, 1 & 3) concerning the etiology of yellow fever.

The following are the essential clinical data:—A.O., 34 years, bullfighter, Spanish, was admitted to the lazaret in Mérida on August 5, 1910. Febrile symptoms had been present since the day before, and the patient, on his arrival, complained of intense headache, nausea, and slight abdominal pain of no certain localization. These symptoms continued, though less severe, during the patient's stay at the lazaret. He never felt seriously ill, even when the temperature was comparatively high. The fever was of an intermittent type, as shown in the accompanying chart (I), the maximum always occurring during the afternoon or night. Repeated examinations of the blood demonstrated the absence of malarial parasites. No hepatic or other organic affections were detected. The urine contained, on the fourth day of the disease, a trace of albumin and a few hyaline casts and gave a strong indican-reaction, but on other occasions no abnormal elements were found, especially no bile-pigments. Jaundice was not observed.

A differential leucocyte count on the eighth day of the disease gave the following result:—Polymorphonuclears 49·25 %, large mononuclears and transitionals 18·5 %, lymphocytes 32 %, eosinophiles 0·25%.

The patient left the lazaret before the fever had subsided, but he appears to have eventually recovered and to have left the town shortly afterwards.

CHART I



Malarial fever was suspected, but, as no malarial parasites were found, no quinine was given to begin with, because the possibility of yellow fever had also to be considered, the patient being non-immune. Strong objections are made by most practitioners, in Yucatán as elsewhere, to the use of quinine in yellow fever. From the seventh day of the disease, however, 1 gram of quinine was given daily, without any apparent effect on the temperature.

On two occasions, on the fourth and eighth day, there were observed in Giemsa preparations of the blood (dry method) elements as those shown in Plate XXIV, figs. 1-5. They were fairly numerous on the fourth day, but scarce on the eighth, and absent on the ninth and tenth day.

These elements show a somewhat faintly stained body and a darker spot, which is, as a rule, situated in or near the periphery of the body. The faintly stained portion may be supposed to represent the cytoplasm, though its colour is a pale purple, and not the characteristic blue which is generally observed in protozoa; the dark red spot has the aspect of chromatin. Some slight variations are observed as to the shape and size of the cytoplasm, but, as a whole, the elements are fairly uniform. Many of the elements are apparently intracorpuscular, but it is difficult to say, whether they are really situated inside or on the surface of the erythrocytes. Others are extracorpuscular, either isolated or, as in fig. 5, forming groups. The largest diameter of the bodies is about 0·7-1·1 μ.

The aspect of these bodies seems to indicate their being parasites; this impression was also that of Professor Nuttall, who very kindly examined one of the slides with me. Other possibilities are that they might represent nuclear granules or blood platelets. With regard to the first possibility it may be noted that one or two nucleated erythrocytes were observed in several of the slides, but no transitions were seen between such nuclei and the bodies described. The presence of a well-differentiated body (cytoplasm) besides the chromatin is also a strong argument against this possibility. With regard to blood platelets, such were seen in all parts of the specimens, also in the vicinity of the supposed parasitic bodies, but they differed entirely from the latter in structure. In fact, the blood platelets present showed no peculiar morphological features when compared with those in other blood smears, stained according to the same technique.

The well-defined structure of the bodies seems to me to exclude the possibility that they might be cocci.

Evidently there is no question of confusion with malarial parasites. The bodies show a slight resemblance with *Babesia* and probably also with Theiler's (1910, 1 & 2) *Anaplasma marginale*, though in the latter apparently no cytoplasm is discernible,

according to Theiler, and also to Sieber (1911), and they are not unlike the 'yellow fever bodies' which I have described and believe to be the parasite of that disease, and for which I have recently proposed the name of *Paraplasma flavigenum* (1911, 3). They differ, however, from these bodies in being nearly uniform in size and shape, whilst the *Paraplasma flavigenum* shows considerable variations. Other differences are that the present bodies are largely extracorpuscular and that their cytoplasm does not show a clear blue staining after Giemsa, as is often, though not always, seen in the case of the yellow fever bodies. In the latter, again, double chromatin spots are frequent, but have not been observed in the present bodies.

Moreover, the disease in which they were found differed considerably from the ordinary clinical picture of yellow fever. We know, of course, that yellow fever may reveal different characters, and it has to be considered that the patient was a non-immune in a yellow fever country, but I would not be inclined to accept this case as one of yellow fever without absolutely convincing proofs.

A number of cases of unclassified fevers were observed in Yucatán by other physicians as well as by myself, and it appears not at all unlikely that yellow fever may be one of a group of diseases produced by blood-inhabiting parasites, which may be different, but more or less intimately related to each other. Similar differences might, perhaps, account for the differences in the clinical pictures of yellow fever in various countries, to which I have recently called attention (1911, 2), and thus account also for some of the obscure points in the epidemiology of yellow fever.

Should this hypothesis prove correct, the case here described would probably belong to the same group of diseases, and its parasite be another species of the same genus as the yellow fever parasite. In this case a suitable name would be *Paraplasma subflavigenum*.

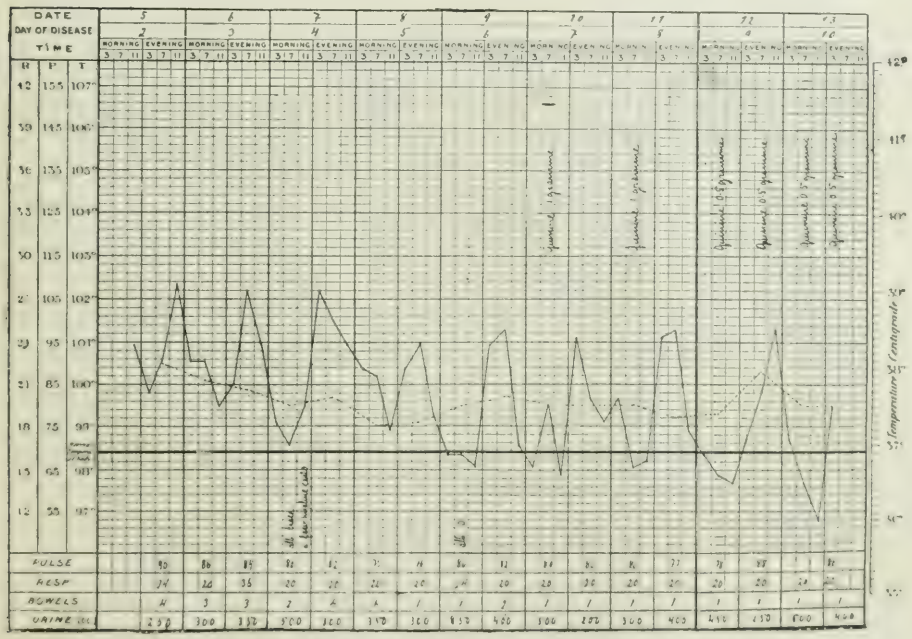
A type specimen has been deposited in the collection of the Liverpool School of Tropical Medicine.

For the clinical history I am indebted to Drs. Canto and Vargas, who kindly invited me to see the patient with them.

II.

In the blood of a monkey (*Ateles sp.*) which I had kept for some time in my laboratory in Mérida, Yucatán, I found an intracorpuscular parasite, of which types are shown in figs. 6-8 of Plate XXIV. The monkey had been subjected to other experiments, but not to any inoculations of blood, nor to intravascular injections. It presented an irregular fever (see Chart II) for which the experiments undertaken did not seem to account. When the blood parasites had been found once, repeated examinations showed their presence on several occasions.

CHART II



The parasites were never numerous, and sometimes very scarce. The only forms observed were rings with one or two chromatin granules as shown in the plate, neither large schizonts nor gametes were found. Puncture of the spleen resulted in the finding of a small number of pigmented leucocytes (fig. 9) besides a few schizonts similar to those observed in the peripheral blood.

This observation is, of course, incomplete, but the forms observed were very similar to young schizonts of *Plasmodium praecox*, and the parasite probably belongs to the genus *Plasmodium*. Similar parasites in apes have been described by Kossel (1899), Lühe (1906), Dutton, Todd and Tobey (1906), Halberstädter and v. Prowazek (1907), Mayer (1907, 1908), Flu (1908), and Gonder and Berenberg-Gossler (1908).

III.

A disease is observed in Yucatán in imported cattle, which is known as yellow fever of the cows. I had the opportunity of examining blood smears from two such cases, and found small intracorpuscular parasites, apparently a species of *Babesia*. No drawings were, however, made at that time, and my preparations have now faded to a considerable degree. The forms observed were small solid protoplasma bodies with a single chromatin spot or ring-shaped bodies with two or three chromatin granules. In the smallest forms the protoplasm was extremely scarce, or seemed entirely absent, so that only a chromatin spot was seen. No division forms were observed.

Clinically, the disease was characterized by fever, jaundice, oliguria and diarrhoea. The urine was only examined once, in a fatal case on the day before death; it contained albumin and bile-pigments, but neither haemoglobin nor blood corpuscles. The disease was said to last for about a week in fatal cases, and anuria to be frequent shortly before death. The mortality was said to be very high.

On a cow which I observed shortly before it died, a few ticks were found, as also on other members of the same herd. A specimen was sent to Professor Nuttall, who kindly informed me that it belonged to the species *Boophilus australis*, and that it had been embodied in his catalogue under the number 1019.

IV.

Trypanosoma lewisi was found in about 50 % of rats examined.

V.

Malarial parasites were found in sixty-nine cases examined in the laboratory, besides in some private cases. The species observed has been stated in fifty-one cases. *Plasmodium vivax* was present alone in nine cases, *P. malariae* in two, and *P. praecox* in 37; *P. praecox* was seen together with *P. vivax* in two cases, and together with *P. malariae* in one case.

No case of malaria seemed to have originated inside the city proper, and in one case only did it seem probable that infection had taken place in a suburb, all the other patients had been exposed to infection in the country or in other places.

REFERENCES

Dutton, J. E., Todd, J. L., and Tobey, E. N. (1906). 'Concerning certain parasitic protozoa observed in Africa.' Liverpool Sch. Trop. Med. Memoir XXI, pp. 87-97.

Flu, P. C. (1908). 'Untersuchungen über Affenmalaria.' Arch. f. Protistenk., XII, pp. 323-330.

Gonder, R., & Berenberg-Gossler, H. v. (1908). 'Untersuchungen über Malariaplasmodien der Affen.' Malaria, I, pp. 47-56.

Halberstädter, L., & Prowazek, S. v. (1907). 'Untersuchungen über die Malariaparasiten der Affen.' Arb. a. d. Kais. Ges., XXVI, pp. 37-43.

Kossel, H. (1899). 'Über einen malariaähnlichen Blutparasiten bei Affen.' Zeitschr. f. Hyg. u. Infektionskrankh., XXXII, pp. 25-32.

Lühe, M. (1906). 'Die im Blute schmarotzenden Protozoen.' etc., in Mense's Handbuch der Tropenkrankheiten, III, Leipzig.

Mayer, M. (1907). 'Über Malaria beim Affen.' Med. Klin., No. 20.

——— (1908). 'Über Malariaparasiten bei Affen.' Arch. f. Protistenk., XII, pp. 314-322.

Seidelin, H. (1909). 'Zur Aetiologie des gelben Fiebers.' Berl. klin. Woch., No. 18, pp. 821-823.

——— (1910). 'Experiences in Yucatán.' Journ. Trop. Med. Hyg., XIII, pp. 535-540.

——— (1911, 1). 'Protozoon-like bodies in the blood and organs of yellow fever patients.' Journ. Path. and Bacteriol., XV, pp. 282-288.

——— (1911, 2). 'Some differential leucocyte counts in yellow fever cases.' Yell. Fev. Bur. Bull., I, 3, pp. 109-126.

——— (1911, 3). 'The Etiology of yellow fever.' Yell. Fev. Bur. Bull., I, 7, pp. 229-258.

Sieber, H. (1911). '*Anaplasma marginale*.' Rep. Govern. Vet. Bact., Union of S. Africa. Pretoria, pp. 104-116.

Theiler, A. (1910, 1). '*Anaplasma marginale*.' (Genus nov. et species nov.) Bull. Soc. Path. Exot., III, pp. 135-137.

——— (1910, 2). 'Texasfieber, Rotwasser u. Gallenkrankheit der Rinder.' Ztschr. f. Infekt. paras. Krankh. u. Hyg. d. Haustiere, VIII, pp. 39-62.

EXPLANATION OF PLATE XXIV

The figures have been drawn by Mrs. Margrethe Seidelin with Abbé's camera lucida. Zeiss. Apoch. Obj. Imm. 3 mm., Comp. oc. 12.

Figs. 1-5.—Parasites from human blood. × 1300.

Figs. 6-8.—*Plasmodium* sp., from blood of *Ateles* sp. × 1400.

Fig. 9.—Pigmented leucocyte (transitional form) from spleen of *Ateles* sp. × 1400.

1

2

3

4

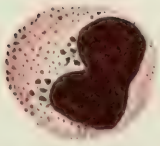
5

6

7

8

9



THE GENUS *PRISTIRHYNCHOMYIA*, BRUNETTI, 1910

BY

CAPTAIN W. S. PATTON, M.B., I.M.S.

AND

CAPTAIN F. W. CRAGG, M.D., I.M.S.

THE KING INSTITUTE OF PREVENTIVE MEDICINE, GUINDY, MADRAS

(*Received for publication* 20 *November*, 1911)

The genus *Pristirhynchomyia* was created by Brunetti to include a single species, *lineata* (Brunetti, 1910), which he separated from the genus *Philaematomyia*, Austen, on account of certain differences in the proboscis. The following is a portion of his original description of the genus:—

'With the exception of an important modification of the proboscis, identical with *Philaematomyia*, Aust., the general characters, the venation and chaetotaxy agreeing exactly.

'The two parts of the proboscis, however, are structurally reversed, the wide basal part being fleshy and flexible, the second part (of about equal length) being sub-cylindrical, black and distinctly chitinized, possibly retractile to the extent of its withdrawal partly or wholly within the fleshy basal portion. At the end of the chitinous portion is a soft fleshy tip, the terminal orifice being of the shape of a triangle with a rounded base (the edges being thickened somewhat by a rim bearing the teeth). At the apex of the triangle is a single black tooth, whilst arranged around the orifice above are three pairs of similar black teeth.

'Under high microscopic power the apparent "rim" of the orifice is seen to be the base of each tooth extended considerably on each side, so that the "rim" is not continuous.

'The new genus is intermediate between *Philaematomyia* and *Musca*, but the presence of the teeth suggests that it can hardly be other than a "biting fly."'

We have examined several preparations of the mouth parts of this fly, including one made from a co-type, identified by Brunetti, for which we are indebted to Dr. Annandale, Superintendent of the Indian Museum, and have found that the description quoted above, and the figures which accompany it, are inaccurate and misleading, and that such deviations as there are from the type of *Philaematomyia* are differences in degree and not in kind. The proboscis is not 'structurally reversed' but consists of a proximal portion, the rostrum, containing the fulcrum, and a distal portion, the haustellum, which bears the oral lobes; that is to say, the proboscis is of the ordinary muscid type, and is as retractile as that of *Musca domestica*, Lin. There are five pairs of teeth (one pair of which is rudimentary), and not three as stated by Brunetti. The 'rim' is not formed by the bases of the teeth, but corresponds to the discal sclerite of non-blood-sucking muscids. It is obvious, from the terms used in the description, that the writer of it is not familiar with the structure of the proboscis of a typical *Musca*.

We infer from Brunetti's paper that the description was written after examination of pinned specimens and proboscides mounted in Canada balsam without special manipulation. Under such conditions it is extremely difficult, in fact impossible, to make out the finer details of the parts. To study the chitinous structures satisfactorily it is essential to clear the preparations in potash, and to mount in balsam in varying positions. In the case of this fly, one can, with a little care, dissect off the chitinous ring to which the teeth are attached, and mount it flat.

The proboscis of this fly closely resembles that of *Philaematomyia insignis*, Austen, which will shortly be described in detail by one of us (F.W.C.). All the structures found in the latter fly are represented in *lineata*, and it will only be necessary here to indicate the points of difference between the two. The proximal portion, or rostrum, is relatively somewhat larger than in *insignis;* the distal part, or haustellum is, contrary to what one would infer from the original description, considerably less densely chitinized, and therefore less rigid. The theca is shallower, and the thickening of its lateral margins not nearly so well marked. The middle portion of the membrane which stretches between the two

lateral borders of the theca is not chitinized into a definite 'labial gutter' such as one finds in *Philaematomyia insignis* and *Stomoxys*; in place of this there are two rod-like thickenings, between which the membrane is only slightly chitinized, the whole forming a trough to accommodate the labrum-epipharynx and hypopharynx.

The labellar rods, which are the lateral arms of the discal sclerite, are articulated, as in *insignis*, on the ends of the labial rods. The main portion of each is conical, the thickest part lying in front of the end of the labial rod, the sharp internal angles projecting inwards towards one another at the level of the tip of the labrum. The upper ends of the rods are pointed, and diverge widely from one another. The lower and outer angle of each of these wedge-shaped rods is produced downwards and inwards, and directly downwards at the tip, where it projects beyond the axial apophysis. This downward prolongation gives attachment to the inner ends of the teeth.

The *axial apophysis* is **V**-shaped, its pointed apex forming the apex of a triangle, from the sides of which the teeth appear to arise. It is situated, however, posterior to the downward prolongation of the labellar rods, and is not directly connected with the teeth. The proximal ends of its arms are attached to the labellar rods on their posterior surface.

The *teeth* resemble those of *insignis*, but are considerably more slender and pointed. They arise from the membrane between the pseudo-tracheae by expanded bases, the inner ends of which are elongated and attached to the downward prolongation of the labellar rods. The second, third and fourth teeth on each side are approximately equal in size. The fifth is smaller, but similar, while the first pair, which lie on either side of the tip of the axial apophysis, are about a quarter the size of the others, and project very little from the surface of the membrane. The 'serrated blades' of *Philaematomyia insignis* are represented by four pairs of spine-bearing chitinous strands. These arise from the membrane between the base of the teeth, a little away from the distal portion of the labellar rods. Each runs outwards parallel to the teeth, and bifurcates in **U**-shaped manner at the level of the most distal portion of the attachment of the teeth to the membrane. At the point of bifurcation the lateral arms split up into three or four filaments,

which lie to a certain extent super-imposed on one another, and are somewhat difficult to see.

The *Pseudo-tracheal membrane* presents no peculiarities, being identical with that of *insignis*, except that the channels are a little wider. The fourth to the seventh channels, counting from the front, terminate between the lateral arms of the spine-bearing strands, the filaments arising from the strand lying parallel to the horseshoe-shaped chitinous rings of the pseudo-tracheal channels.

From the foregoing it will be seen that this fly corresponds in all essential particulars to Austen's description of the genus *Philaematomyia*, and we are of opinion that it should be placed in that genus, since we think that it is unjustifiable to create new genera on minor details of structure which cannot be made out without dissection.

One of us (W.S.P.) has bred *Philaematomyia lineata* from the egg. Its breeding habits are identical with those of *insignis*, shortly to be described by us. From thirty to forty eggs are laid in cow dung, all in one place. The eggs are slightly smaller than those of *insignis*, but are otherwise similar. The larvae behave in the same way when about to pupate, and have the same lemon yellow colour. The puparium is similar but smaller.

Brunetti states that Dr. Annandale has frequently seen this fly distended with blood, while feeding on cattle. We have not ourselves observed this, although this fly is fairly common here during the colder months. The fact that it has been taken distended with blood is, of course, no proof that it can obtain blood independently; it may, like *Musca pattoni*, Austen, and *Musca convexifrons*, Thomson, suck up the blood which exudes from the wounds made by other biting flies.

The male fly, which Brunetti does not appear to have seen, is much like the female, but has a distinctly lighter abdomen. It will be described fully on another occasion.

Note

Since writing the above, one of us (W.S.P.) has found a new species of *Philaematomyia*, the habits of which are identical with those of *insignis*. It is distinguished by its large size (0·7-0·8 cm.

long) and its coloration. There are the usual four admedian dark stripes on the thorax, and a narrow median dark line on the dorsal surface of the abdomen; the lateral halves of each segment are a light olive green colour. The proboscis resembles that of *insignis*, but has five large teeth and two rudimentary ones on each side. We propose naming this fly *Philaematomyia gurnei*, sp. nov., after Mrs. Patton, who was one of the first to see it. A detailed description will be published later.

REFERENCES

Austen, E. E. (1909). New Genera and Species of bloodsucking Muscidae from the Ethiopian and Oriental Regions in the British Museum. Ann. Mag. Nat. His. 8, iii, p. 285.

Brunetti, E. (1910). Revision of the Oriental bloodsucking Muscidae (*Stomoxinae*, *Philaematomyia*, Aust., and *Pristirhynchomyia*, gen. nov.) Rec. Ind. Mus. IV, 4, p. 59.

Cragg, F. W. (1911). The Structure of the Proboscis of *Philaematomyia insignis*, Austen. (Will be published in the 'Scientific Memoirs Series.')

EXPLANATION OF PLATE XXV

Fig. I.—The proboscis, seen in profile, drawn from a potash preparation.

f. Fulcrum.

M. Membraneous wall of the rostrum.

sl.d. Salivary duct (enclosed within the membrane).

l.ap. Labral apodeme.

p. Palp.

l.ep. Labrum-epipharynx.

hy. Hypopharynx.

lb.r. Labial rod.

l.r. Labellar rod.

fu. Furca.

Fig. II.—The teeth and connected structures, seen from the front, when extended. Drawn from a potash preparation.

ax.p. Axial apophysis.

pt. Pseudo-tracheal channel.

s. Spine-bearing chitinous strand, representing the serrated blades of *Philaematomyia insignis*.

l.r. Labellar rod.

lb.r. Labial rod.

l.ep. Labrum-epipharynx.

t. Teeth.

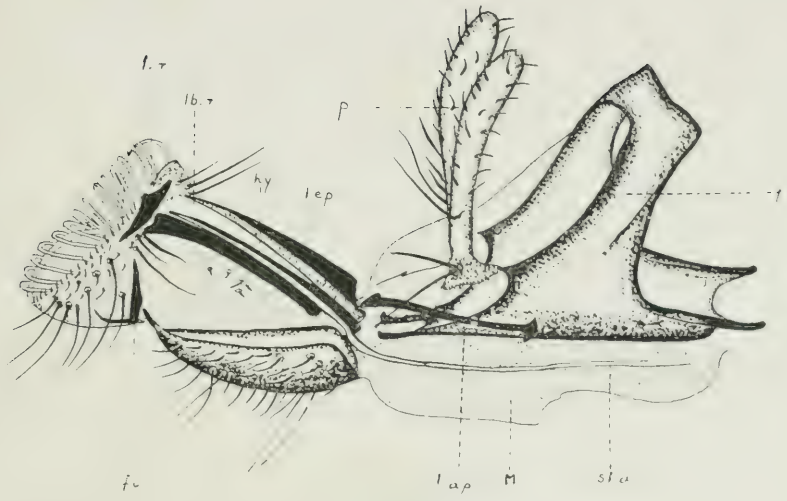


Fig. 1

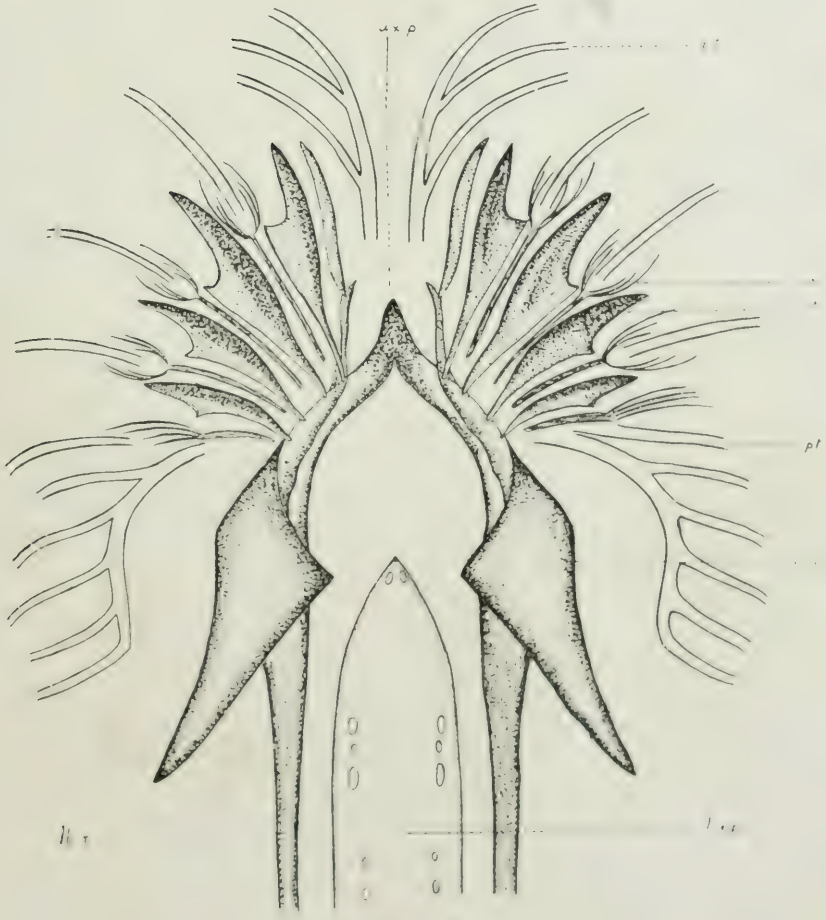


Fig. 2

THE LIFE HISTORY OF *PHILAEMATOMYIA INSIGNIS*, AUSTEN

BY

CAPTAIN W. S. PATTON, M.B. (EDIN.), I.M.S.,

AND

CAPTAIN F. W. CRAGG, M.D. (EDIN.), I.M.S.

THE KING INSTITUTE OF PREVENTIVE MEDICINE,
GUINDY, MADRAS

(*Received for publication 27 November*, 1911)

It is not a little remarkable that, although only two years have elapsed since Mr. Austen described *Philaematomyia insignis* as a new genus and new species, the fly has already been found to be widely distributed throughout the East. It has been recorded from most parts of India, from Ceylon, from Cyprus and also from Central Africa and Socotra. Moreover, a new species, *lineata*, has been described by Brunetti (placed, erroneously, as we believe, in a new genus *Pristirhynchomyia*), and we ourselves will shortly describe another species from Madras, under the name of *Philaematomyia gunnei*. It is extremely probable that the genus is a large and widely distributed one, which has escaped the attention of entomologists on account of its close resemblance to non-blood-sucking muscids. One of us (F.W.C.) will shortly describe the biting apparatus of *insignis*, and it will then be shown that, although the teeth are quite formidable weapons, they are so concealed by the pseudo-tracheal membrane that even in potash preparations a certain amount of dissection is required to expose them. In pinned specimens it is only exceptionally that one can see them.

These flies are of very considerable interest, on account of the well-defined position they occupy in the muscid group. Structurally, they are intermediate between *Stomoxys* and *Musca*, while as regards their habits, they are intermediate between the non-blood-sucking Musca (*M. domestica*, Lin., and *M. nebulo*, Fabr.) and

such flies as *Musca pattoni*, Austen, and *Musca convexifrons*, Thomson, which have no piercing apparatus, and yet feed entirely on blood, sucking up that which exudes from the bites of *Tabanus*, *Chrysops*, *Haematopota* and *Philaematomyia*.

The breeding habits of this fly resemble in general those of non-blood-sucking muscids. The eggs, fifty to sixty in number, are laid in cow dung, the fly appearing to prefer small patches, freshly dropped, rather than larger collections of dried dung. On alighting, the female crawls over the surface until its finds a small crack or crevice; the ovipositor, which is similar to that of *Musca domestica*, is now thrust into the dung, the abdomen being depressed, and all the eggs are deposited in a heap, from 1/8 to 1/4 of an inch below the surface. The process takes from six to ten minutes.

When there are a large number of flies about, one often sees half a dozen or more all depositing their eggs in the same spot, their ovipositors being close to one another, while their heads are turned outwards. When the flies have finished laying their eggs an irregular heap of several hundreds will be found just beneath the surface. The eggs are laid from early morning until noon, rarely later.



Fig. 1—Egg.

The egg (Fig. 1) measures from 2 to 2·2 mm. long by 0·4 mm. broad. It is of the usual muscid shape, an elongated ovoid, gently convex along one margin and concave on the other; one end is slightly more pointed than the other, but it has no spine. It is a yellowish white colour and densely opaque. On the concave margin there is a shallow groove, difficult to distinguish at the pointed end, but widening out towards the broader end of the egg.

The larvae hatch out, through the groove, in from eight to nine hours, that is, on the evening of the day on which the eggs were laid. When mature, they measure about 1·25 cm., their greatest

breadth being about one-seventh the length. They are cylindrical, pointed at the oral end, and are composed of twelve segments, of which the posterior seven are of approximately equal size. They are bright lemon yellow in colour, and on this account are readily distinguished from other muscid larvae. All the larvae remain together up to the evening of the second day, and then migrate,



Fig. 2.—Larva.

still in a company, from the dung, passing out from its under surface, and burrow in the ground, under leaves, etc., to pupate. This habit of migrating together is somewhat remarkable, and has not been observed in any other of the Muscids we have studied here.

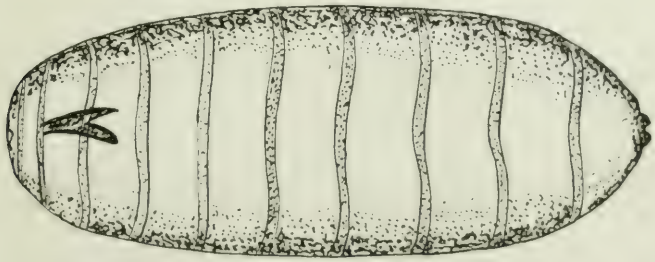


Fig. 3.—Pupa.

The *puparium* resembles that of Musca. It measures, on the average, 0·5 cm. long by 0·18 broad, though there is a considerable amount of variation in this respect. It is of a light mahogany colour, and eleven segments can be distinguished. At the posterior end there are two conspicuous kidney-shaped spiracles, raised somewhat above the surface; these have characteristic markings, as indicated in the figure, which are conspicuous on account of their orange colour, and which are distinctive of the species.

Breeding takes place in Madras throughout the year. The total time occupied is from six to seven days, varying a little according to the temperature. The large size of the egg and the short time in which it hatches suggest that the eggs undergo some development before they are laid.

About eight hours after hatching the fly is ready to feed. Both sexes suck blood, which forms their main if not their only food, though we have seen them rubbing their proboscides on the surface of cow dung when about to lay their eggs, in a manner which strongly suggests that they suck up the juices from the surface, possibly using their teeth to penetrate the crust.

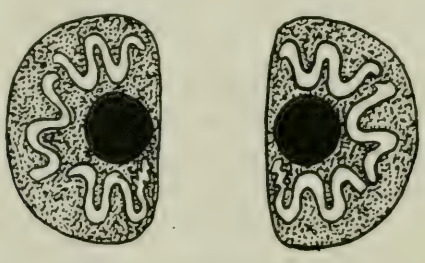


Fig. 4.—Posterior Spiracles.

They feed almost exclusively on cattle and, as far as we have observed, they only occasionally bite human beings. They do not ordinarily exhibit any preference for any particular part of the skin of the host, though we have found them especially attached to the abdomen of calves which have been shaved for vaccination. When feeding, the fly lies closely pressed against the skin of the host, its body being parallel with the surface. They remain until fully gorged, and are not easily disturbed; they can, in fact, be easily picked off with the fingers. Like most blood-suckers, they pass out a clear watery fluid, and later apparently unaltered blood, from the anus as the abdomen distends.

From the somewhat lethargic habit of the fly, and from the fact that it breeds rapidly and (in Madras) throughout the year, one would expect to find that it has natural enemies, which keep down its numbers. The chief of these is a small Hymenopteron (not yet identified). The habit of this wasp is to settle on the dung and to

watch for a fly laying its eggs. Having marked a victim, it crawls up to within an inch or two of it and then makes a short rapid flight, settles on the fly, and after stinging it through the head carries it away, holding it by means of its sting and its hind legs. We have seen as many as five of these wasps on the same patch of dung. The fly, busily engaged in laying its eggs, usually falls a ready victim to its extremely active enemy; should it escape the first assault, and fly away, the wasp will follow it and either catch it as it settles on a blade of grass, or later when it returns to the dung. The wasp also frequently attacks flies while feeding on cattle. Unfortunately, the wasp is so small and flies so rapidly that we have been unable to follow it to its nest.

Several small species of spider also prey on *Philaematomyia*, catching them while laying their eggs. There is also a small Asilid which has the same habit, and can often be seen to swoop down on a fly and carry it off, grasped in its forelegs, to a neighbouring twig, where it sucks out its juices.

Lastly, we have for several years observed a small *Tachinid*, which rests on a blade of grass close to a piece of dung in which *Philaematomyia* is laying its eggs. Its behaviour is very remarkable and suggestive. It sits with its head directed towards the fly, and every now and then darts towards it, in a very direct and business-like manner, and at once returns to its perch. It certainly does not catch, or attempt to catch, the fly. We have dissected specimens of this Tachinid caught in the act, and have found that its ovaries contain well-developed larvae, enclosed in thin transparent membranes, through which one can see the larva making active butting movements, as if in the endeavour to free itself. The fly, like most of the members of that family, deposits larvae and not eggs, and we suspect that the presence of this Tachinid is associated with that of the Hymenopteron mentioned above, and that the Tachinid deposits its larva on the *Philaematomyia* with the intention that it shall be carried to the nest of the wasp, where it would find ample food. We hope in time to settle this interesting biological problem.

The simplest way of breeding *Philaematomyia insignis* is to watch for a number of flies laying their eggs, and then to scoop up the whole patch of dung and place it in a long tin tray about

two to four inches deep. The dung should be placed at one end of the tray and a quantity of sand at the other. The larvae, when about to pupate, will migrate to the sand, from which the pupae can be removed to a closed vessel.

REFERENCES

1. Austen, E. E. (1909). Ann. Mag. Nat. Hist. (8), III, 295.
2. Brunetti, E. (1910). Revision of the Oriental bloodsucking Muscidae. Rec. Ind. Mus., IV, 4, p. 59.
3. Cragg, F. W. The Structure of the Proboscis of *Philaematomyia insignis*, Austen. (Will shortly be published in the Scient. Mem. Med. Off. India, Calcutta.)
4. Patton, W. S. & Cragg, F. W. The Genus *Pristirhynchomyia*. Ann. Trop. Med., V, 4, pp. 509-514.

THE MEASUREMENTS OF A THOUSAND EXAMPLES OF *TRYPANOSOMA VIVAX*

BY

B. BLACKLOCK, M.D.

From the Runcorn Research Laboratories.

(*Received for publication* 24 *January*, 1912)

The trypanosomes which form the basis of this communication are derived from a strain kept up in goats, which strain was obtained from a horse naturally infected in the Gambia. In a previous paper dealing with the trypanosomes found in this horse, Yorke and Blacklock (1911) acknowledged their indebtedness to Professor Todd for his kindness in sending the animal (*Horse A*) to Runcorn. As was stated in the above paper, two forms of parasites were present in the blood of *Horse A*, viz., a comparatively long, free-flagellated trypanosome, possessed of great activity of movement, and a short non-flagellated form, of sluggish movement. It is with the former of these trypanosomes, the long form, that this present paper deals, and the name *T. vivax* is used to include *T. cazalboui*, which it will be recalled Bruce (1910) says is probably the same species.

THE SPECIES OF ANIMAL HOST CHOSEN

In making drawings and measurements of a particular trypanosome, it appears desirable that all observers should, if possible, adhere to the same species of animal host in order to establish a ready standard for purposes of comparison. For this reason it would have been of advantage if one could have utilised one of the smaller laboratory animals, for example the white rat, which has proved itself so easily susceptible to many forms of trypanosome infection, and by means of which ready comparisons can be made between *T. gambiense*, *T. brucei*, *T. rhodesiense* and many other trypanosomes. But as regards the long parasite with which we are dealing, it was found to be a matter of difficulty to produce infection in small laboratory animals. In fact, a few

rabbits and white rats alone, of a large number of experimentally inoculated animals, became infected, and the majority even of those few recovered. So great was the difficulty encountered in this respect that it was thought better to make use of goats, in which animals infection was produced with great ease and certainty, and in which after the first few passages, the long parasite alone persisted. The short non-flagellated parasite of *Horse A* had died out in the goats, as shown not only by microscopic examination of the blood, but also by repeated inoculations (with negative results) into a large number of small laboratory animals.

GENERAL PLAN OF MEASUREMENT

The trypanosomes were drawn and measured in small groups, each containing twenty specimens. The number of goats from which parasites were measured was four, which, for convenience, are called A, B, C, D. The number of days of the disease represented is twenty-two. There are thus fifty groups, each of which contains twenty trypanosomes, drawn and measured from four goats, on twenty-two days. The earliest day of the disease represented among the goats is the seventeenth, and the latest is the forty-fifth.

The arrangement of the groups is made as follows:—

(1) 400 trypanosomes (twenty groups of twenty each) were drawn on twenty separate days of the disease from three of the goats (A, B, C).

(2) 400 trypanosomes (twenty groups of twenty each) were drawn on one day of the disease from one goat (D).

(3) 100 trypanosomes (five groups of twenty each) were drawn on one day of the disease from one goat (C.)

(4) 100 trypanosomes (five groups of twenty each) were drawn on one day of the disease from one goat (D).

The reasons for adopting this plan are these:—

(1) By spreading out the first 400 trypanosomes over three goats and twenty days of disease, and confining the second 400 trypanosomes to one goat and one day of the disease, one can compare two large sets of parasites drawn and measured under widely different conditions.

(2) By drawing and measuring two sets, each of 100 trypanosomes, drawn on a single day of the disease

from separate goats, one can form comparisons between small numbers on a somewhat different basis.

(3) Finally, one can collect for comparison other sets of 100 or less spread over various animals and various days and compare them with those given above, and can form tables and charts to illustrate the comparative relation of any one set to another, larger or smaller.

METHOD OF FIXING, STAINING AND DRAWING

Thin films, made from the blood of the ear, were dried, fixed for five minutes in absolute alcohol, and stained with Giemsa's stain for twenty minutes*. Non-dividing parasites (taken in order as they were found) were drawn in clear outline with the help of the Abbé camera lucida, using a No. 18 Zeiss compensating ocular with a 2 mm. apochromatic objective.

METHOD OF MEASURING

Measurements were carried out by Stephens's method, which is briefly as follows: Along the middle of a narrow strip of smooth transparent paper a straight line is drawn. It is convenient to have this line considerably longer than the longest trypanosome to be measured, and terminating short of the margin of the paper. At one extremity of the line a mark is made for identification. A sharp pin or mounted needle is then taken, and the marked end of the straight line is made to coincide with one extremity of the outlined trypanosome to be measured. Transfix the end of the line to the extremity of the subjacent trypanosome, with the needle held perpendicularly. Rotate the paper until the straight line lies along the long axis of the trypanosome. Hold the paper in position with one hand and with the other take out the needle and pass it through the tissue paper again at the first point at which the axis of the trypanosome begins to deviate from the straight line. Repeat this process, following carefully every bend of the trypanosome and keeping in the long axis of it until the opposite extremity is reached. Hold the needle steady and place against it

* Two drops of Giemsa's solution added to each cubic centimetre of distilled water. A subsequent rapid wash with 10 per cent. orange tannin solution gave good results for drawing purposes.

a millimetre scale and read off the distance from the needle to the marked starting point of the straight line. By this simple method a very accurate measurement of the drawn parasite is obtained, and from it, by calculation, the actual length of the trypanosome.

CONSIDERATION OF THE RESULTS OBTAINED

An analysis of the 1,000 trypanosomes and of the component sets is given in Table I. From this table it will be seen that the average measurement of the 1,000 dealt with is 21·7 μ, the range being from a maximum trypanosome of 26·7 μ long to a minimum of 15·5 μ long. The trypanosome is monomorphic, all forms found being provided with a well-marked free flagellum.

Between the averages of the two sets of 400 each, there is a difference of only 0·9 μ, the first set averaging 21·4 μ and the second 22·3 μ.

Between the averages of the two sets of 100 each a smaller difference is observed, the first averaging 20·9 μ, the second 21·5 μ. When one comes down to the groups of twenty each, larger variations are naturally observable.

In Table II the trypanosomes are tabulated according to their percentage in microns under three heads, viz., those measuring less than 20 μ, those between 20 μ and 23 μ, and those measuring 23 μ and over.

It will be seen from this table that in each set dealt with, whether 1,000, 400, or 100, the largest number of trypanosomes constantly lies between 20 μ and 23 μ.

In Charts I, II, III, which give, for the various groups, a graphic representation of the percentages in length, this same fact is clearly shown.

CONCLUSIONS

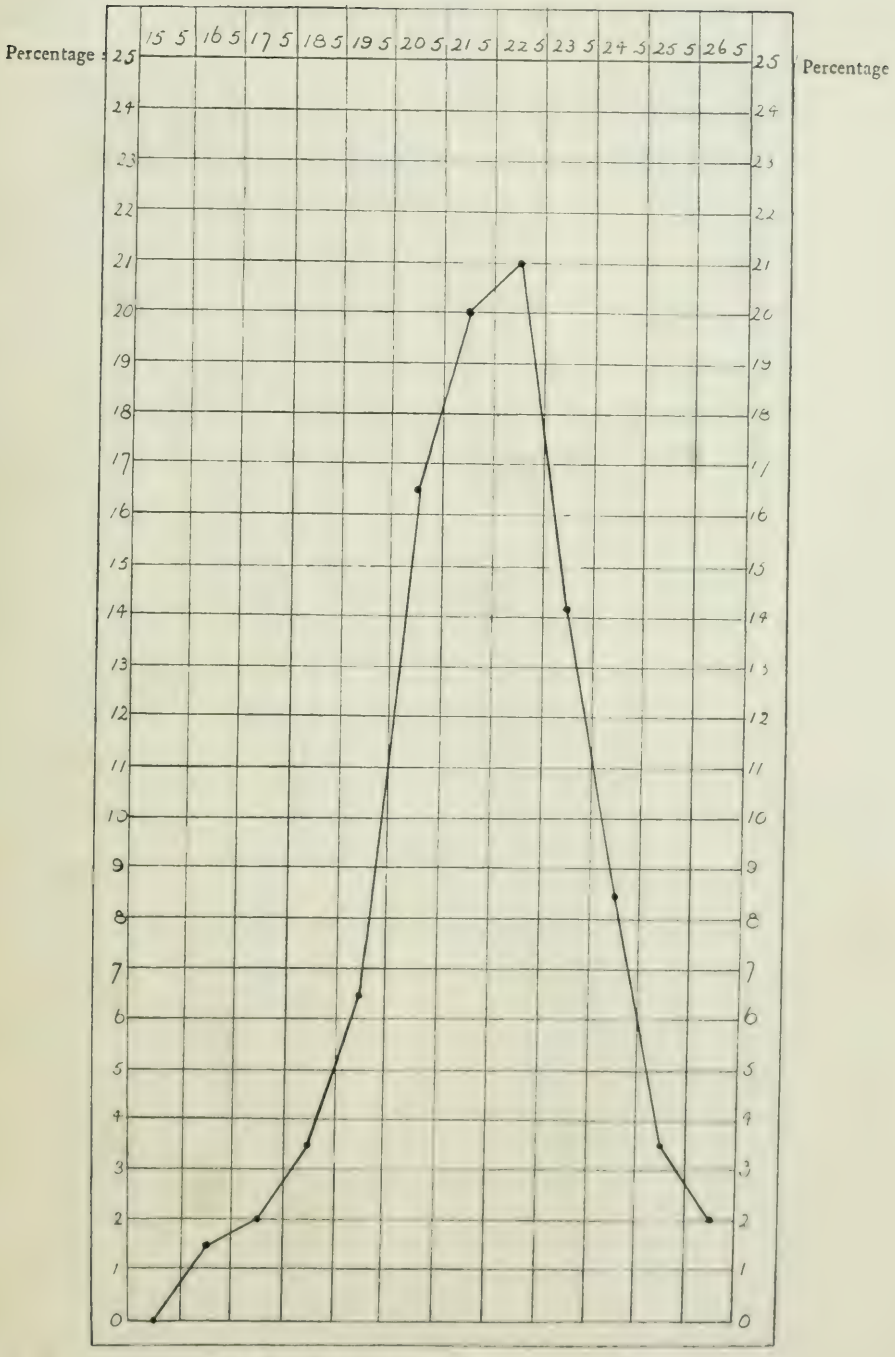
(1) This *T. vivax* from the Gambia (*Horse A*) is a free flagellated monomorphic trypanosome of an average length of 21·7 μ.

(2) The range of its extreme measurements is comparatively small.

(3) The curve of percentage length is remarkably constant, whether large or small numbers are dealt with.

CHART I

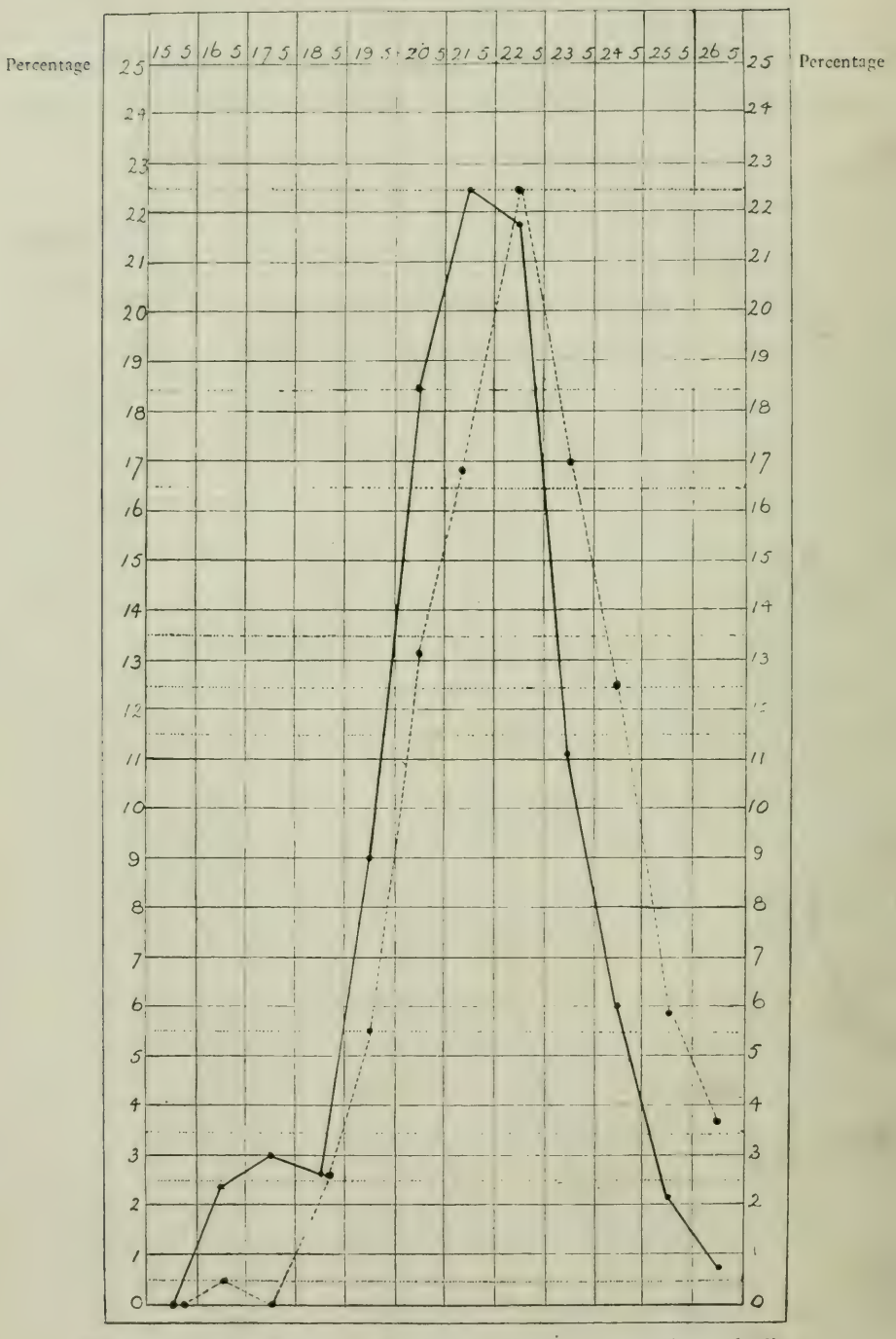
Length in Microns



1000 *Trypanosoma vivax* measured on 22 days in 4 Goats.

CHART II

Length in Microns

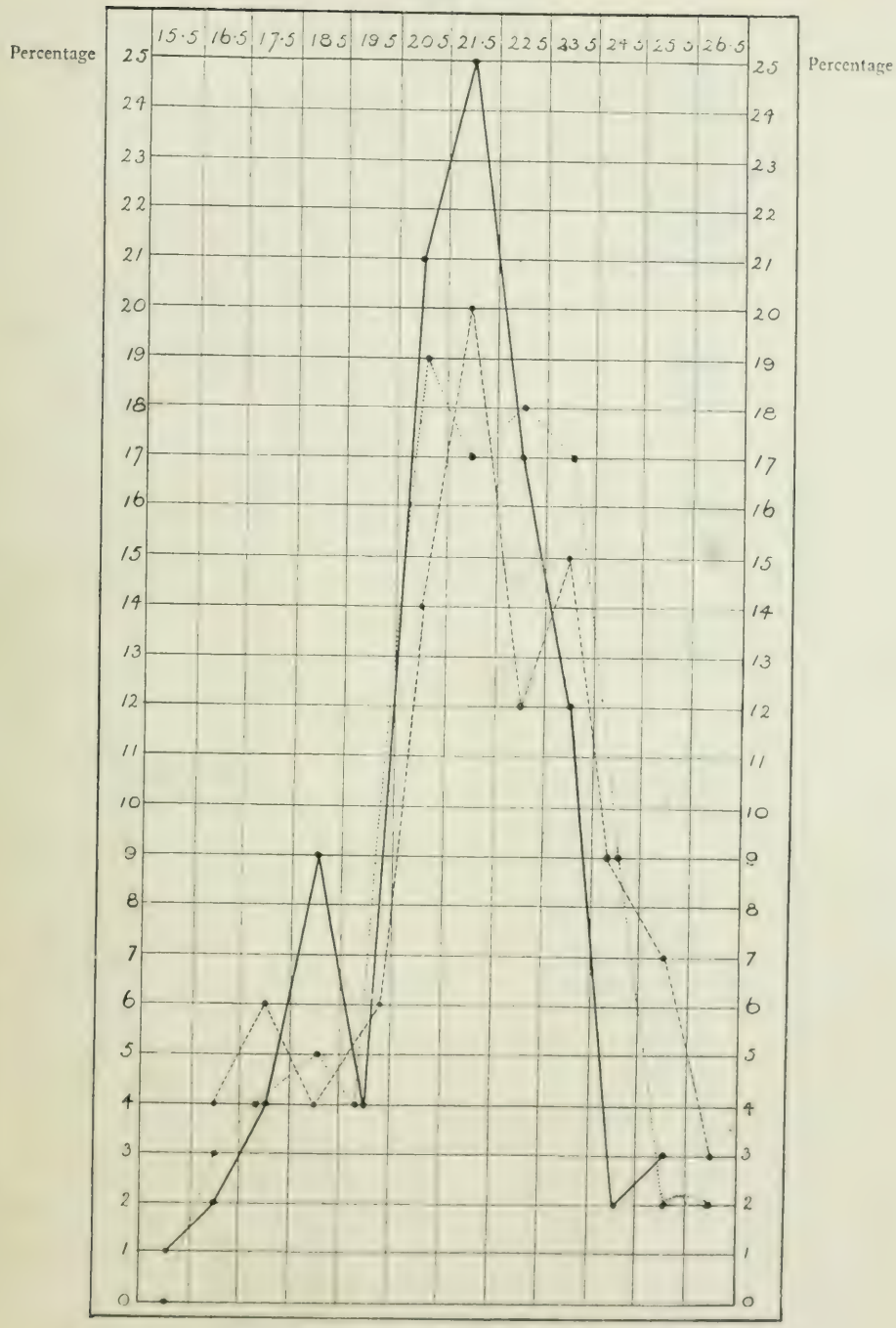


400 *Trypanosoma vivax* measured from 3 Goats A B C on 20 days of the disease.

400 ,, ,, ,, ,, 1 Goat D on 1 day ,, ,,

CHART III

Length in Microns



——— 100 *Trypanosoma vivax* measured in Goat C on 1 day of the disease.

..... 100 ,, ,, ,, ,, D ,, ,, ,,

- - - - - 100 ,, ,, ,, in 4 Goats A B C D on 5 days of the disease (chosen at random)

TABLE I. Analysis of 1000 examples of *T. vivax* in goats, drawn and measured

Number of trypanosomes measured	Number of animals represented	Number of days represented	Maximum trypanosome measured	Minimum trypanosome measured	Average measurement of total number drawn	Highest average in any group of 20	Lowest average of any group of 20	The shortest maximum trypanosome in any group of 20	The longest minimum trypanosome in any group of 20
1000 composed as under	4 A B C D	22	26·7	15·5	21·7	23·2	18·8	22·5	21·5
400	3 A B C	20	26·6	16·0	21·4	23·0	18·8	22·5	20·4
400	1 D	1	26·7	16·0	22·3	23·2	20·6	24·0	21·5
100	1 C	1	25·2	15·5	20·9	21·8	20·6	23·5	17·5
100	1 D	1	26·5	16·0	21·5	22·1	20·9	24·2	20·0

TABLE II. Showing percentage incidence according to length in microns of 1000 *Trypanosoma vivax*, and of the groups composing the total.

Number of trypanosomes	Number of goats from which drawn	Number of days of disease represented	PERCENTAGE OF (1) Trypanosomes measuring less than 20μ	(2) Trypanosomes measuring between 20 and 23μ	(3) Trypanosomes measuring 23μ and over
1000 composed as under	4 A B C D	22	13·8	57·8	28·4
400	3 A B C	20	17·2	62·7	20·1
400	1 D	1	8·5	52·5	39·0
100	1 C	1	20·0	63·0	17·0
100	1 D	1	16·0	54·0	30·0
For comparison 100 5 groups of 20 (taken at random)	4 A B C D	5	20·0	46·0	34·0

REFERENCES

Bruce and others (1910). 'Trypanosome diseases of domestic animals in Uganda. III, *T. vivax* (Ziemann).' *Proc. Roy. Soc.*, B, Vol. LXXXIII, p. 15.

Yorke and Blacklock (1911). 'The trypanosomes found in two horses naturally infected in the Gambia.' *Annals of Trop. Med. & Parasitol.*, Dec., V, 3, pp. 413-434.

ENUMERATIVE STUDIES ON *T. BRUCEI* IN RATS AND GUINEA-PIGS, AND A COMPARISON WITH *T. RHODESIENSE* AND *T. GAMBIENSE*

BY

JOHN GORDON THOMSON, M.A., M.B., CH.B.

(*Received for publication* 8 *February*, 1912)

This investigation was undertaken while I was assistant to Sir Ronald Ross, under the Sir Edwin Durning-Lawrence Research Fund. Already enumerative studies have been conducted by H. B. Fantham and J. G. Thomson in animals infected with *T. rhodesiense* and *T. gambiense* (1910-11), and a comparison was made between these. It was pointed out that *T. rhodesiense* was more virulent than *T. gambiense*, as shown by the fact that rats and guinea-pigs lived a shorter period when infected with the former, and it was also demonstrated that the incubation period was shorter and the period between the heights of the crests shorter in the cases of rats infected with *T. rhodesiense*.

The following table, based on the results of Fantham and Thomson (1910), shows this difference clearly:—

	No. of Rats examined	Incubation period average, in days	Average duration of life	Period between the heights of the crests
T. rhodesiense ...	22	2·9 days	11·3 days	3-4 days
T. gambiense ...	11	4·4 days	13·8 days	4-6 days

It is possible, therefore, apart from morphology, to distinguish *T. rhodesiense* (Stephens and Fantham, 1910) from *T. gambiense* provided a sufficient number of animals, preferably rats and guinea-pigs, are inoculated with both strains. As a matter of fact, however, the morphology in the case of *T. rhodesiense* in rats, as

pointed out by Stephens and Fantham (1910), makes the diagnosis of *T. rhodesiense* very easy indeed.

I now publish four charts of animals infected with *T. brucei*. The method employed has already been described by D. Thomson (1911). It is necessary in the case of heavily-infected animals to dehaemoglobinise the slide, and spread the film over a large area.

By means of a special pipette (D. Thomson, 1911) a count was made regularly every twenty-four hours of the number of parasites per c.mm. of blood, and these daily counts have been represented diagrammatically in the charts accompanying this paper.

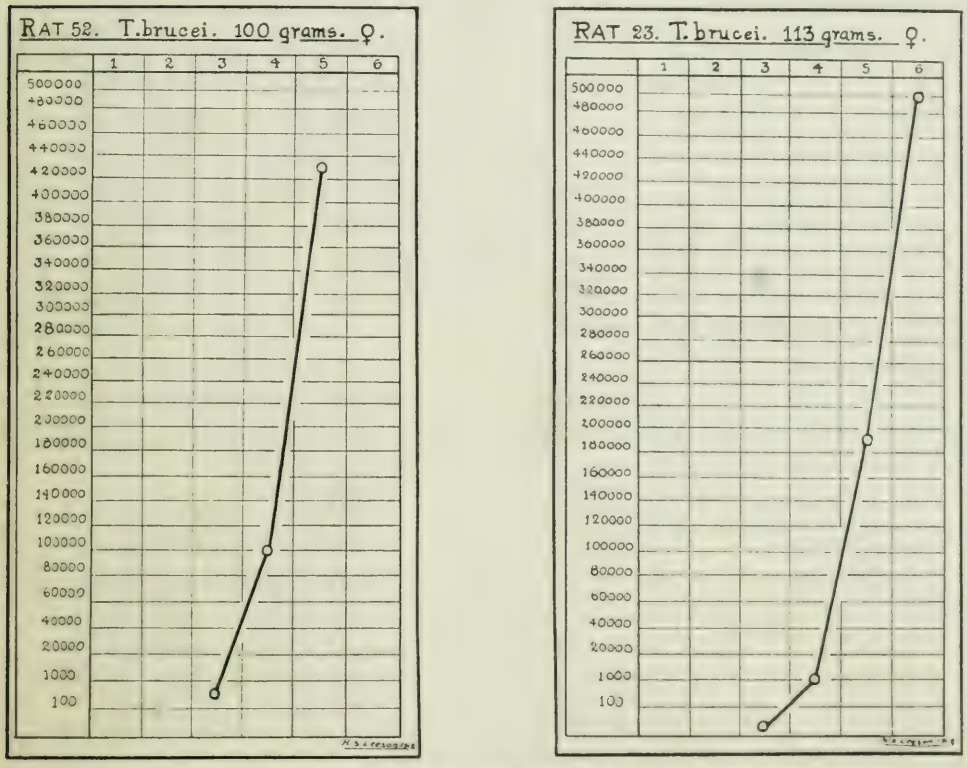
We shall first examine the charts of Rats 52 and 23 (*T. brucei*). Here we note the very rapid multiplication of the parasites. In the case of Rat 52 the parasites rose with a rush from about 1,000 per c.mm. to 430,000 per c.mm. in forty-eight hours, and the animal succumbed. Again, in the case of Rat 23 the parasites rose from under 100 per c.mm. to 490,000 per c.mm. of blood in seventy-two hours, and the animal died shortly after. These extraordinary numbers were never reached in so short a period either in the case of *T. rhodesiense* or *T. gambiense*. In the case of *T. rhodesiense* I have found in a rat treated with atoxyl, and which lived fifty-one days, that the parasites on the fifty-first day rose to one million and a half per c.mm. (R. Ross and J. G. Thomson, 1911), but never in the case of either *T. rhodesiense* or *T. gambiense* have I found the parasites in rats reach such high numbers in forty-eight to seventy-two hours as in the case of *T. brucei*.

Again, it is to be noticed that no periodic variation took place in rats infected with *T. brucei*. The rise was always continuous in the animals observed by me. In the case of rats inoculated with *T. rhodesiense* and *T. gambiense*, H. B. Fantham and J. G. Thomson pointed out that a continuous rise of parasites may take place until death in both (e.g., Rats 18-22, 30-33), but that periodic variation also takes place in both (e.g., Rats 1-17, 23-29). So far, in the strain of *T. brucei* used here, I have been unable to obtain periodic variation in rats, but it is quite possible that such may occur if we could find rats of sufficient resistance.

The rats inoculated with *T. brucei* all died within an average period of six days. When this is compared with the average life of rats inoculated with *T. rhodesiense* and *T. gambiense* we have

a very marked difference. We conclude, therefore, in rats that *T. brucei* is much more virulent than either *T. rhodesiense* or *T. gambiense* as evidenced by three points, namely:—

1. Duration of life.
2. Rapidity of development of parasites.
3. Periodic variations.



GRAPHS SHOW THE DAILY RECORD OF PARASITES FOUND PER C.MM. OF PERIPHERAL BLOOD.

Of course *T. brucei* is essentially an animal trypanosome, and so might be expected to be more virulent to rats than strictly human trypanosomes sub-inoculated into them.

We shall now examine the charts of the two guinea-pigs infected with *T. brucei*.

Here again, when we study the disease in *T. rhodesiense* and *T. gambiense*, we find that the disease runs a more or less chronic

course. In the case of *T. rhodesiense* the average life of the guinea-pig was fifty-nine days, whereas those infected with *T. gambiense* was 111 days. The period between the crests of the waves was longer than in rats, namely, five to eight days (H. B. Fantham and J. G. Thomson, 1910).

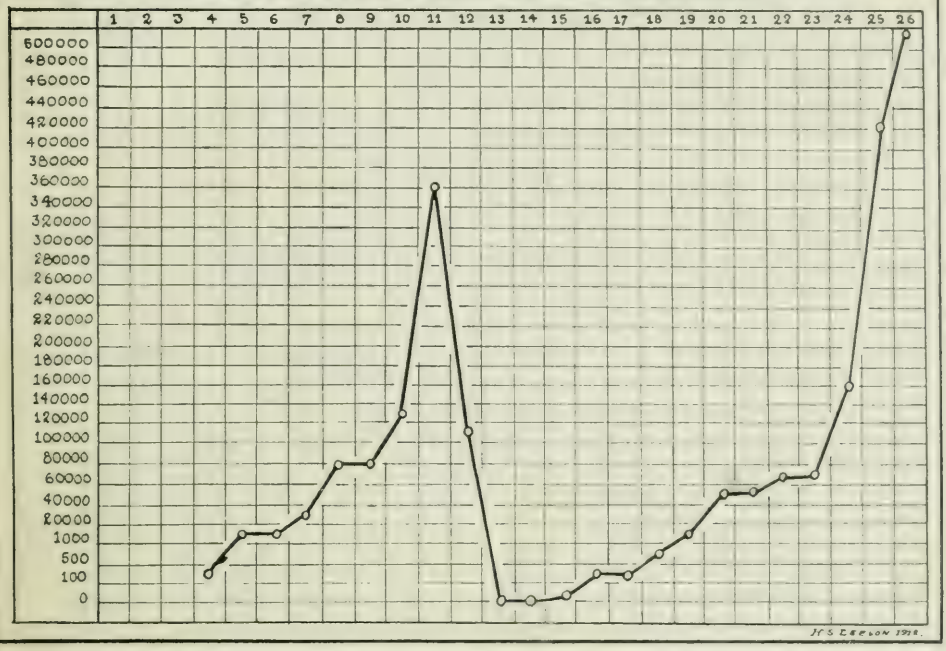
Referring now to the charts of Guinea-pigs 56 and 67, infected with *T. brucei* (see charts), we find that the longest period of life was twenty-six days, and we find again that in the case of *T. brucei* the multiplication of the parasites was of much greater rapidity than in the case of either *T. rhodesiene* or *T. gambiense*. Thus in the case of Guinea-pig 56 (*T. brucei*) the parasites in eighteen days numbered 450,000 per c.mm., and during that time the rise was more or less continuous. There was only one slight fall during that time, which occurred on the twelfth and thirteenth days. The animal died in twenty days. In the case of Guinea-pig 77 (*T. brucei*) we find that on the eleventh day the parasites rose to about 350,000 per c.mm. and then fell steadily for two days, and no parasites were found on the thirteenth or fourteenth days, and only one or two were seen on a film on the fifteenth day. On the sixteenth day the parasites again reappeared and increased steadily for eleven days until they reached over 500,000 trypanosomes per c.mm. of blood. These numbers far exceed those found in either *T. gambiense* or *T. rhodesiense*. The chart of Guinea-pig 77 is of very great interest, as it shows a distinct periodic variation, with a period of about sixteen days between the heights of the crests. This is interesting because of the fact that it shows on the eleventh day the animal was able to survive a very heavy infection, the crisis being reached and a natural recovery taking place for two days. We had evidently resistant forms left (*cf.* Fantham, 1911).

For a permanent cure we must aim at the destruction, therefore, of these resistant forms. In short, if we compare *T. brucei* with *T. rhodesiense* and *T. gambiense* in guinea-pigs we have the following points of difference:—

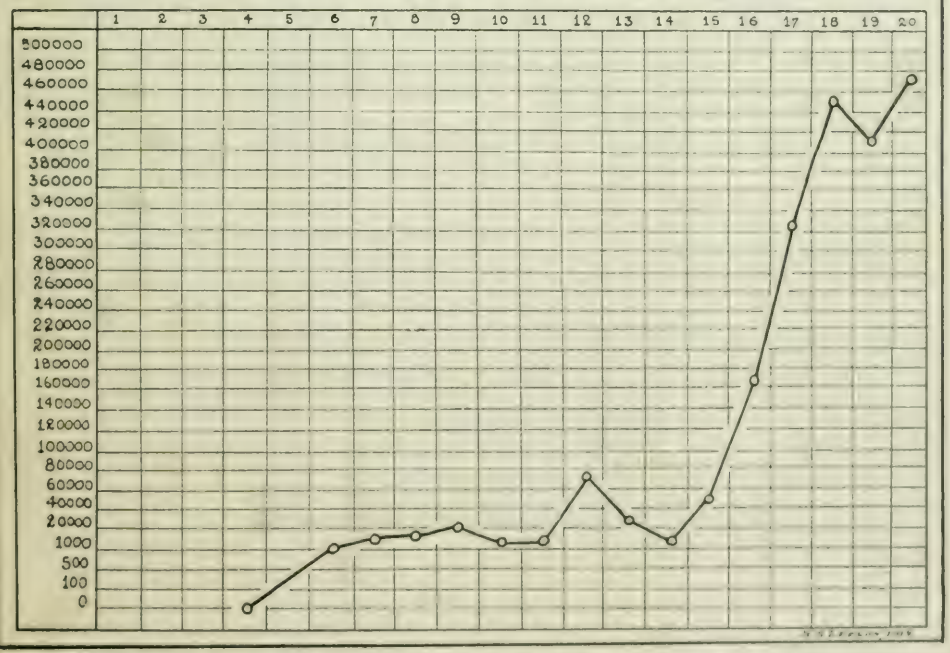
1. *T. brucei* kills guinea-pigs much more rapidly than *T. rhodesiense* or *T. gambiense*.

2. The multiplication of parasites is much more rapid, and they reach much higher numbers in the peripheral blood than in either *T. rhodesiense* or *T. gambiense*.

GUINEA-PIG 77, ROUGH-HAIRED. *T. BRUCEI*. 474 GRAMS. ♂.



GUINEA-PIG 56, *T. BRUCEI*. 742 GRAMS. ♀.



GRAPHS SHOW THE DAILY RECORD OF PARASITES FOUND PER C.MM. OF PERIPHERAL BLOOD.

3. The periodic variations in *guinea-pigs* infected with *T. brucei* is very different from that which takes place in the case of infections with *T. gambiense* and *T. rhodesiense*.

REFERENCES

Fantham and Thomson, J. G. (1910-11). 'Enumerative Studies on *Trypanosoma gambiense* and *T. rhodesiense* in Rats, Guinea-pigs, and Rabbits; Periodic Variations disclosed.' Proc. Roy. Soc., B, Vol. LXXXIII, pp. 206-211 (Prelim. Note); also Ann. Trop. Med. and Parasitol., IV, pp. 417-463.

Fantham, H. B. (1911). 'The life-history of *Trypanosoma gambiense* and *T. rhodesiense* as seen in Rats and Guinea-pigs.' Proc. Roy. Soc., B, Vol. LXXXIII, pp. 212-227; and Ann. Trop. Med. and Parasitol., IV, pp. 465-485, 1 plate.

Ross, R., and Thomson, J. G. (1911). 'Experiments on the Treatment of Animals infected with Trypanosomes by means of Atoxyl, Vaccines, Cold, X-rays, and Leucocytic Extract. Enumerative methods employed.' Proc. Roy. Soc., B, Vol. LXXXIII, pp. 227-234 (Prelim. Note); also Ann. Trop. Med. and Parasitol., IV, pp. 487-527.

Stephens, J. W. W., and Fantham, H. B. (1910). 'On the peculiar morphology of a Trypanosome from a Case of Sleeping Sickness, and the Possibility of its being a new species (*T. rhodesiense*).' Proc. Roy. Soc., B, Vol. LXXXIII, pp. 28-33, 1 plate.

Thomson, D. (1911). 'A new blood-counting pipette for estimating the numbers of leucocytes and blood parasites per cubic millimetre.' Ann. Trop. Med. and Parasitol., V, pp. 471-478.

A NOTE ON THE MEASUREMENTS OF *TRYPANOSOMA VIVAX* IN RABBITS AND WHITE RATS

BY

B. BLACKLOCK, M.D.

From the Runcorn Research Laboratories

(*Received for publication* 8 *February*, 1912)

Using the strain of *T. vivax* previously mentioned (*vide* p. 521) a large number of experimental inoculations into laboratory animals was made. Details of these will form part of a future paper dealing more fully with further work done on the trypanosomes infecting two horses from the Gambia previously referred to. This note deals only with four rabbits and two white rats, which were inoculated from goats which presented a pure infection with *T. vivax*. In the case of the rabbits the incubation period varied from eight to sixteen days, and parasites were present in the peripheral blood for periods varying from one day (the shortest) to ten days (the longest). In the two rats the incubation period was five days, and parasites were found in the peripheral blood for only one day. The greatest number of parasites found in the fresh preparation was, in the case of the rabbits, five to a field (objective DD, ocular No. 4), in the case of the rats one to thirty fields.

The trypanosomes presented great activity of movement in fresh preparations, and in stained preparations in dry films gave the measurement results which are given below. As the rats had trypanosomes in the peripheral blood on one day only, and in small numbers, only a small number of specimens (fifty) could be drawn from one rat. For comparison with these, fifty were drawn from one of the rabbits on one day of the disease.

In the Rabbit. The average length of the fifty trypanosomes is 20·8 μ, the maximum parasite measuring 23·2 μ, and the minimum 17·4 μ.

In the Rat. The average length of the fifty trypanosomes is 21·1 μ, the maximum parasite measuring 26 μ, and the minimum 15 μ.

Table to show percentage incidence according to length in microns of fifty *Trypanosoma vivax* in a rabbit and fifty in a rat.

No. of Trypanosomes	Animal	Percentage of		
		Trypanosomes measuring less than 20μ	Trypanosomes measuring between 20μ and 23μ	Trypanosomes measuring 23μ and over
50	Rabbit	22	72	6
50	Rat	22	58	20

A CASE OF MALARIAL FEVER, SHOWING A TRUE PARASITIC RELAPSE, DURING VIGOROUS AND CONTINUOUS QUININE TREATMENT

BY

SIR RONALD ROSS, K.C.B., F.R.S.

AND

DAVID THOMSON, M.B., CH.B., D.P.H.

(*Received for publication* 12 *February*, 1912)

In the 'Annals of Tropical Medicine and Parasitology,' Vol. V, No. 3, December 30, 1911, we described the occurrence of pseudo or non-parasitic relapses in 6·7 % of our cases of malarial fever, during active quinine treatment of ten grains thrice daily. We were unable to prove that these 'pseudo-relapses,' which usually took the form of a sudden and isolated rise of temperature, had any connection with the original fever, as similar inexplicable rises of temperature occurred in 17 % of other diseases, during treatment in hospital.

Cases of malarial fever, resistant to quinine treatment, and which showed relapses during the treatment have been reported to occur in the Amazon region. During our experience of two years of careful observations on cases of malaria, we found no case which showed any resistant tendency to quinine, and twenty of these cases had contracted fever up the River Amazon. We, therefore stated in our paper (1911) that it was possible that these so-called resistant cases might have been cases of pseudo-relapses, especially as no data had been given with regard to the finding of parasites in them. We are now able, however, to confirm these reports, having observed carefully a case which showed marked resistance to quinine, and which showed a true parasitic relapse during treatment with that drug.

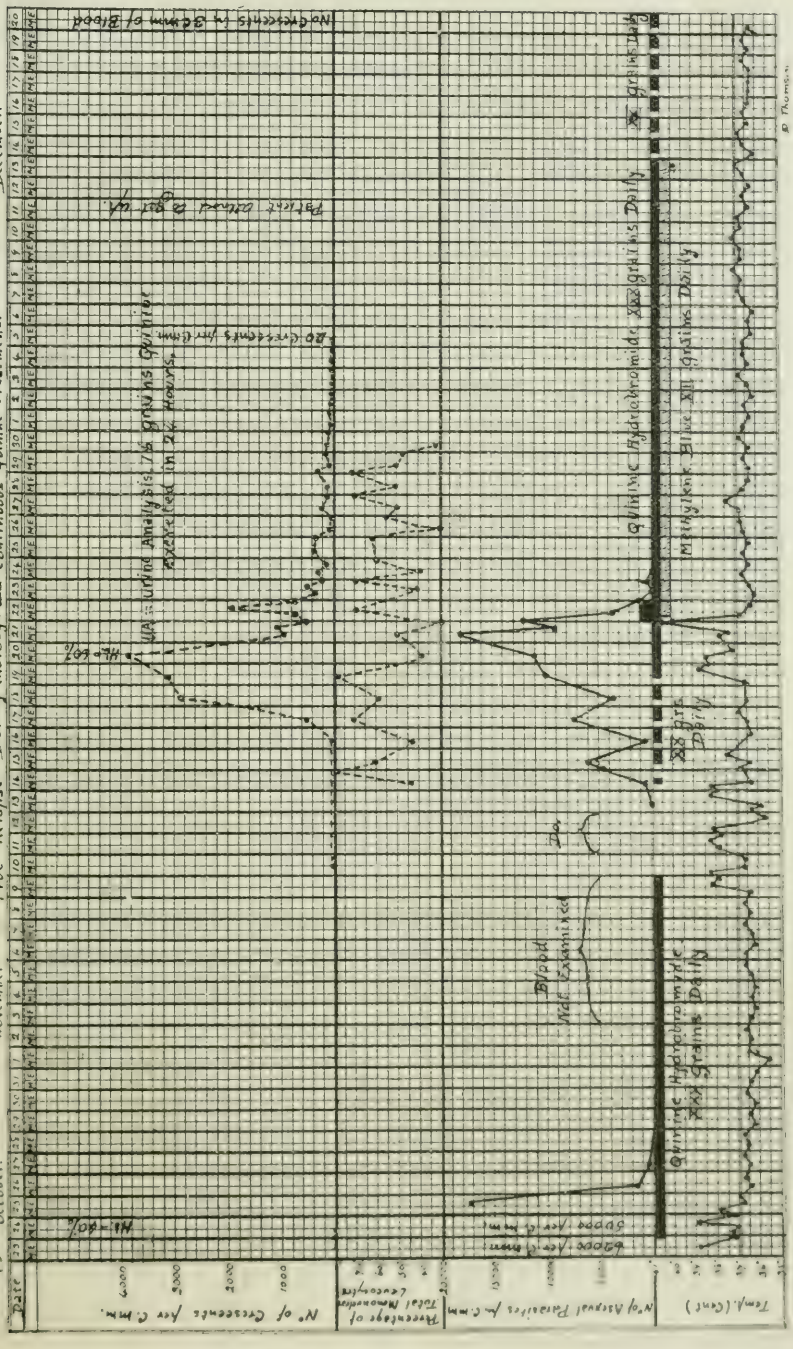
The details of this remarkable case, of which we publish a chart, are as follows:—

Patient E. E., age 65. Half-caste, born in Canada. Occupation, seaman.

History prior to admission. Patient had been to sea for forty-five years, and had sailed to most parts of the world, including India, Japan, etc. He had a slight attack of malarial fever six years ago, but it did not trouble him much. In May, 1911, patient sailed up the Amazon river for the first time. He arrived at Porto Vellho on 17th May, 1911. There he left the ship and took a post ashore as foreman of a gang of labourers. He got an attack of fever on the 2nd of June, and was very ill, and states that he had dysentery as well. He was in bed for a month and was getting quinine thrice daily all the time. After this he remained well for two months, during which period he had quinine, according to his own statement, 'only once in awhile.' He had a second attack of fever in September, but this attack was not quite so severe. He then sailed down the river to Manaos and took a ship to England. He was ill during the whole of this voyage and got quinine only once or twice a week. He was admitted to the Tropical Ward of the Royal Southern Hospital, under our care on the 23rd October, 1911. The duration of his illness before admission was, therefore, 159 days.

Details of case after admission. These are shown graphically on the accompanying chart. On admission patient had fever, and the blood examination revealed a mixed infection of benign and malignant tertian malaria. A few crescents were present (about 16 per c.mm.) The blood showed marked auto agglutination of the red cells and nucleated and stipuled basic red cells were numerous. The haemoglobin was only 40%. There was no appreciable enlargement of the liver or spleen. The patient was very weak and somewhat emaciated, and had a tendency to be slightly delirous and incoherent in his speech.

Quinine hydrobromide in liquid form was administered in doses of ten grains thrice daily, by mouth. This reduced the asexual parasites to below the detectable limit in thin films, in five days, that is, about two days longer than usual. This dosage of quinine was continued for seventeen days, during which period the temperature remained normal. From the 3rd till the 9th November the blood was not examined; on the 10th November, however,


E.E. age 65 P.falciparum + P.vivax. (Amazon.)
True Relapse During thorough and Continuous Quinine Treatment.
1929 October. November December.
Date
Temp. (Cent.)
No. of Asexual Parasites per c.mm.
Percentage of Total Mononuclear Leucocytes
No. of Crescents per c.mm.
Quinine Hydrobromide 20 Grains Daily
Blood not Examined
Do
Methylene Blue XII Grains Daily
Quinine Hydrobromide XX Grains Daily
No Crescents in 20 mm³ of Blood
Parasites Entirely Disappeared
No Crescents per c.mm. Found in 24 Hours.
Malignant Tertian Analysis. 10 Grains Quinine
P. Thomson

a rise of temperature having been noticed, the blood was examined rapidly and no parasites were observed. Thinking that the temperature was one of those pseudo-relapses which we had noticed to occur before, during quinine treatment, we stopped the administration of quinine for a few days. As the temperature, however, persisted and showed a true malarial type, the blood was again examined carefully from the 14th November onwards. Asexual parasites, malignant and benign, were found, as indicated on the chart, and, moreover, crescents began to appear. Quinine was again given, as before, on the 14th November, and the patient by this time was very ill and slightly delirious, and seemed to have difficulty in articulation. He commenced to pass his urine involuntarily. On the 21st November, the fever showed no signs of abating, and on the 22nd November, therefore, thirty grains of quinine bihydrochloride were injected intramuscularly in addition to the usual thirty grains of quinine hydrobromide given by mouth. In addition to this, twelve grains of methylene blue were given daily in pill form. This combined treatment reduced the asexual parasites below the detectable limit in three days, and the crescents were reduced to 20 per c.mm. in fourteen days. The patient improved very rapidly, and was no longer confined to bed after the 10th December. On the 13th December the methylene blue was stopped and the quinine reduced to twenty grains daily. He left hospital on the 20th December.

Urine anaylsis. On the supposition that the quinine may not have been properly absorbed from the digestive tract, on the 21st November a twenty-four hours' specimen of the urine was examined by Dr. G. C. Simpson to estimate the quantity of quinine excreted. It was found to contain sixteen grains. The patient was, therefore, excreting thirteen grains daily out of the thirty grains administered daily by the mouth. This is about the usual amount and showed that the quinine administered was being efficiently absorbed.

A twenty-four hours' specimen of urine was again examined by Dr. Simpson on the 20th December, during treatment with twenty grains daily. The amount recovered in the urine was six grains. This patient was, therefore, absorbing his quinine efficiently, so that we are forced to conclude that this case of malaria (mixed infection) showed a most unusual resistance to thorough and continuous quinine treatment.

Before concluding we would like to remark that, though this patient was a weak old man of 65, yet in spite of a practically continuous treatment with quinine (thirty grains daily) for fifty days, he had no symptoms of deafness, nor did he complain of any of the symptoms of quininism. It has been our practice to give every case of malaria coming under our care ten grains of quinine thrice daily for a period of three weeks, and out of 200 cases treated in this manner, during the past two years, we have had few or no complaints of quininism produced by this so-called severe treatment. During such treatment the patients improve in health most markedly. They gain weight rapidly, and the haemoglobin percentage rises very quickly. They have always been able to hear well, and during convalescence they were able to work well in the ward. It is, however, not advisable to inform the patient as to the quantity of quinine that is being administered. It would appear that the majority of patients felt no more inconvenience from doses of thirty grains daily, than from doses of ten grains daily, after the first few days of administration.

CONCLUSIONS

(I.) A case of malaria, mixed infection (malignant tertian and benign tertian), contracted at Porto Vellho, River Amazon, has come under our care, shewing most unusual and marked resistance to quinine, and also a true parasitic relapse during thorough treatment with that drug.

(II.) Patients can tolerate, with very little discomfort, much more quinine than is generally supposed, especially when they are not aware that they are receiving heroic treatment.

REFERENCES TO LITERATURE

Ross & D. Thomson (1911). 'Pseudo-Relapses in Cases of Malarial Fever during Continuous Quinine Treatment.' Ann. Trop. Med. and Parasitol., Dec., V, 3, pp. 409-412.

THE UNIVERSITY PRESS OF LIVERPOOL

Publications of the Liverpool School of Tropical Medicine

MEMOIR I

Malarial Fever: its Cause, Prevention, and Treatment (1903). Containing full Details for the use of Travellers, Sportsmen, Soldiers, and Residents in Malarious Places. By RONALD ROSS, C.B., F.R.S., F.R.C.S. With Frontispiece and Plate. 8vo. 2s. 6d.

MEMOIR II

Report of the Malaria Expedition to Sierra Leone (1899). By RONALD ROSS, D.P.H., M.R.C.S., H. E. ANNETT, M.B., D.P.H., and E. E. AUSTEN. Being a full account of the first expedition of the School, and containing besides much matter relating to the parasites of malaria, to the gnats which carry them, and embodying some previous observations of Major Ross in India. Illustrated by four maps and five full-page collotypes. Quarto. Price 21s. nett.

MEMOIR III

Report of the Malaria Expedition to Nigeria (1900). By H. E. ANNETT, M.D., D.P.H., the late J. EVERETT DUTTON, M.B., Ch.B., and J. H. ELLIOTT, M.D. Part I. *Malarial Fever*, etc. Giving a full account of the expedition, with numerous views in the text, charts, maps, and two plates, and containing much matter of general importance. Quarto. Price 10s. 6d. nett

MEMOIR IV

Report of the Malaria Expedition to Nigeria (1900). By the same authors. Part II. *Filariasis.* Containing many new observations upon Filariae of Birds, with numerous illustrations and nineteen plates, five of which are coloured and give the microscopical anatomy of the head of *Anopheles costalis* (by the late Dr. DUTTON). Quarto. Out of print.

MEMOIR V, PART 1

First Progress Report of the Campaign against Mosquitoes in Sierra Leone (1901). By Major R. Ross, F.R.C.S., D.P.H., F.R.S., dated 15th October, 1901, giving details of the commencement of the Campaign, with a letter from Dr. DANIELS regarding the results arrived at to date. 8vo. Price 1s.

MEMOIR V, PART 2

Second Progress Report of the Campaign against Mosquitoes in Sierra Leone. By M. LOGAN TAYLOR, M.B., dated 15th September, 1902. 8vo. Price 1s.

MEMOIR VII

Report of the Yellow Fever Expedition to Para (1900). By H. E. DURHAM, M.B., F.R.C.S., and the late WALTER MYERS, M.B. (Dr. Walter Myers died of Yellow Fever whilst serving on this expedition). Quarto. Out of print.

MEMOIR VIII

Report on the Sanitary Condition of Cape Coast Town (1902). With suggestions as to improvement of same, by M. Logan Taylor, M.B., Ch.B. 8vo. Out of print.

MEMOIR IX.

Report on Malaria at Ismailia and Suez (1903). By Ronald Ross, F.R.C.S., D.P.H., F.R.S., C.B. 8vo. Out of print

MEMOIR X

Report of the Malaria Expedition to the Gambia (1902). By the late J. E. Dutton, M.B., Ch.B. Quarto. Price 15s. nett.

MEMOIR XI

First Report of the Trypanosomiasis Expedition to Senegambia (1902). By the late J. Everett Dutton, M.B., Ch.B., Vict., and John L. Todd, B.A., M.D., C.M., McGill. Quarto. Price 15s. nett.

MEMOIR XII

The Anti-Malaria Measures at Ismailia (1904). By Rubert Boyce, M.B., F.R.S. Price 1s.

MEMOIR XIII

Reports of the Trypanosomiasis Expedition to the Congo (1903-1904). By the late J. Everett Dutton, M.B., John L. Todd, M.D., and Cuthbert Christy, M.D. Quarto. Price 21s. nett.

MEMOIR XIV

Report on the Sanitation and Anti-Malarial Measures in practice in Bathurst, Conakry, and Freetown (1905). By Rubert Boyce, M.B., F.R.S., Arthur Evans, M.R.C.S., H. Herbert Clarke, M.A., B.C., Cantab. Quarto. Eight plates. Price 5s.

MEMOIR XV

General Sanitation and Anti-Malarial Measures in Sekondi, the Goldfields, and Kumassi, and a comparison between the conditions of European residence in India. By Lieut.-Colonel Giles. Quarto. Price 7s. 6d. nett.

MEMOIR XVI

Trypanosomes, Trypanosomiasis, and Sleeping Sickness: Pathology and Treatment. By H. Wolferstan Thomas, M.D., McGill, and Anton Breinl, M.U.Dr., Prag. Quarto. 6 plates (5 coloured) and 7 charts. Price 12s. 6d. nett.

Gland Puncture in Trypanosomiasis. By the late J. Everett Dutton, M.B., Vict., and John L. Todd, B.A., M.D., McGill. Two charts and one figure.

MEMOIR XVII

The Nature of Human Tick-Fever in the Eastern part of the Congo Free State. By the late J. Everett Dutton, M.B., and John L. Todd, B.A., M.D., McGill. Quarto. With map, four plates, and nine temperature charts. Price 7s. 6d. nett.

On the External Anatomy of *Ornithodoros moubata*. By R. Newstead, A.L.S., F.E.S. With two plates.

MEMOIR XVIII

I. Gland Palpation in Human Trypanosomiasis;

AND

II. The Distribution and Spread of 'Sleeping Sickness' in the Congo Free State. By the late J. Everett Dutton, M.B., and John L. Todd, B.A., M.D., C.M., McGill. With four maps (2 colours) and four plates.

III. A New Dermanyssid Acarid. By R. Newstead, A.L.S., F.E.S., and John L. Todd, B.A., M.D., C.M., McGill. With one plate.

IV. Another New Dermanyssid Acarid. By R. Newstead, A.L.S., F.E.S. With one plate.

V. Anatomy of the Proboscis of Biting Flies. By J. W. W. Stephens, M.D., Cantab., and R. Newstead, A.L.S., F.E.S. With six plates.

Imp. 8vo. Price 7s. 6d. nett

MEMOIR XIX

Yellow Fever Prophylaxis in New Orleans in 1905. By Rubert Boyce, M.B., F.R.S. Imp. 8vo. Maps and six plates. Price 5s. nett.

MEMOIR XX

I. La prophylaxie de la Malaria dans les principaux postes de l'État Indépendant du Congo. By the late J. Everett Dutton, M.B., and John L. Todd, B.A., M.D. With four maps and four illustrations.

II. The Animal Reactions of the Spirochæta of African 'Tick Fever.' By Anton Breinl, M.D., and A. Kinghorn, M.B.

III. The Specific Nature of the Spirochæta of African 'Tick Fever.' By Anton Breinl, M.D. Imp. 8vo. Price 5s. nett.

MEMOIR XXI

I. The Runcorn Research Laboratories of the Liverpool School of Tropical Medicine. With five plates.

II. An Experimental Study of the Spirochaete of the African Tick Fever (*Spirochaeta duttoni*). By Anton Breinl, M.U.Dr., and Allan Kinghorn, M.B. With one figure and ten charts.

III. A Note on a New Spirochaete found in a Mouse. By the same authors. With one figure.

IV. Comparison between the Trypanosomes Present by Day and by Night in the Peripheral Blood of Cases of Human Trypanosomiasis. By the late J. Everett Dutton, M.B., John L. Todd, B.A., M.D., C.M., and E. N. Tobey, A.B., A.M., M.D.

V. The Lesions in the Lymphatic Glands in Human Trypanosomiasis. By R. Howard Mole, M.D. With two plates.

VI. Concerning certain Parasitic Protozoa observed in Africa. By the late J. Everett Dutton, M.B., John L. Todd, B.A., M.D., C.M., and E. N. Tobey, A.B., A.M., M.D. With two figures and one coloured plate.

VII. Attempts to Cultivate *Spirochaeta duttoni*. By Lewis A. Williams, M.D., D.P.H., and R. Stenhouse Williams, M.B., D.P.H.

VIII. Attempts to Transmit Spirochaetes by the Bites of *Cimex lectularius*. By Anton Breinl, M.U.Dr., Allan Kinghorn, M.B., and John L. Todd, B.A., M.D. Imp. 8vo. Price 7s. 6d. nett.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. I. No. 1.

Insects and other Arthropoda collected in the Congo Free State. By R. Newstead, A.L.S., F.E.S., the late J. E. Dutton, M.B., and John L. Todd, B.A., M.D., C.M., McGill. Six plates.

Description of Two New Species of African Ticks. By H. Neumann.

On some Parasites in the Museum of the Liverpool School of Tropical Medicine. By A. Looss. Three plates.

The Presence of Spirochaeta Duttoni in the Ova of Ornithodoros Moubata. By Capt. R. Markham Carter, I.M.S. One plate.

A note on the Therapeutics of Trypanosomiasis. By Benjamin Moore, M.A., D.Sc., M. Nierenstein, Ph.D., and John L. Todd, B.A., M.D., C.M., McGill. Imp. 8vo. Price 7s. 6d. nett.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. I. No. 2.

An Automatic Oiler for the Destruction and Prevention of Mosquito Larvae in Cesspools and other collections of Water. By E. H. Ross, and H. C. Ross. One plate.

The Anatomy of the Proboscis of Biting Flies. Part II, Stomoxys. By J. W. W. Stephens, M.D., and R. Newstead, A.L.S., F.E.S. Eight plates.

Trypanosome Transmission Experiments. By the late J. Everett Dutton, M.B., John L. Todd, B.A., M.D., C.M., McGill, and J. W. Hanington, M.D., McGill.

Cattle Trypanosomiasis in the Congo Free State. By the late J. Everett Dutton, M.B., John L. Todd, B.A., M.D., C.M., McGill, and Allan Kinghorn, M.B. Two plates and six charts.

Concerning the Treatment of Experimental Trypanosomiasis. By Benjamin Moore, M.A., D.Sc., M. Nierenstein, Ph.D., and John L. Todd, B.A., M.D., C.M., McGill. Imp. 8vo. Price 7s. 6d. nett.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. I. No. 3.

Concerning certain Parasitic Protozoa observed in Africa. By the late J. Everett Dutton, M.B., Vict., John L. Todd, B.A., M.D. McGill, and E. N. Tobey, A.B., A.M., M.D. Harvard. Thirteen plates.

Yaws. By C. W. Branch, M.B., C.M. (Edin.)

A Description of some Gold Coast Entomostraca. By W. M. Graham, B.A., M.B.

Notes on Dr. Graham's Collection of Cyclopidae from the African Gold Coast. By G. Stewardson Brady, M.D., LL.D., D.Sc., F.R.S. Four plates.

On the Morphology and Life History of *Spirochaeta duttoni*. By Anton Breinl, M.U.Dr. (Prag.) One plate.

The Cytology of the Trypanosomes. Part I. By J. E. Salvin-Moore, A.R.C.S., F.L.S., F.Z.S., and Anton Breinl, M.U.Dr. (Prag.) Five plates.

Imp. 8vo. Price 7s. 6d. nett

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. I. No. 4.

Observations on the so-called 'Canary Fever.' By C. E. Walker.

Contribution à l'étude de *Porocephalus moniliformis*. Par A. Broden et J. Rodhain. One plate.

On the Habits, Life-Cycle, and Breeding Places of the Common House-Fly (*Musca domestica*, Linn.). By R. Newstead, A.L.S., F.E.S. Six plates.

Some Notes on the Morphology of *Spirochæta duttoni* in the Organs of Rats. By J. J. van Loghem, M.D., Amsterdam.

Malaria and History. By W. H. S. Jones, M.A.

Two New Human Cestodes and a New Linguatulid. By J. W. W. Stephens, M.D., Cantab., D.P.H. One plate.

Imp. 8vo. Price 7s. 6d. nett.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. II. No. 1.

Atoxyl and Trypanosomiasis. By Sir Rubert Boyce, F.R.S., and Anton Breinl, M.U.D. Prag.

My Experience of Trypanosomiasis in Europeans and its Treatment by Atoxyl and other drugs. By Sir Patrick Manson, K.C.M.G., F.R.S.

Imp. 8vo. Price 7s. 6d. nett

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. II. No. 2.

Reports of the 'Sleeping Sickness' Expedition to the Zambesi for the years 1907-1908. By ALLAN KINGHORN, M.B., and R. EUSTACE MONTGOMERY, M.R.C.V.S. One map.

A Report on Trypanosomiasis of Domestic Stock in North-Western Rhodesia. By R. EUSTACE MONTGOMERY, M.R.C.V.S., and ALLAN KINGHORN, M.B. One map and ten charts.

Report on the Work of the Greek Antimalaria League during the year 1907. By M. HADJIMICHALIS, President, and JEAN P. CARDAMATIS, General Secretary. Imp. 8vo. Price 7s. 6d. nett

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. II. No. 3.

A Peculiar Intralobular Cirrhosis of the Liver produced by the Protozoal Parasite of Kala-azar. By LEONARD ROGERS, M.D., F.R.C.P., B.S., F.R.C.S., I.M.S. One coloured plate.

What is '*Schistosomum mansoni*' Sambon 1907? By Dr. A. Looss.

The Prevention of Dengue Fever. By E. H. Ross, M.R.C.S. Eng., L.R.C.P. Lond.

The Life History of *Trypanosoma lewisi*. By J. E. SALVIN-MOORE, A.R.C.S., F.L.S., F.Z.S., ANTON BREINL, M.U.Dr. Prag., and EDWARD HINDLE, A.R.C.S. Lond. Four plates.

Notes on the Effects of Therapeutic Agents on Trypanosomes in respect to (*a*) Acquired Resistance of the Parasites to the Drug, and (*b*) Changes in Virulence of the Strains after Escape from the Drug. By BENJAMIN MOORE, M.A., D.Sc. (R.U.I.), MAXIMILIAN NIERENSTEIN, Ph.D. Berne, and JOHN LANCELOT TODD, B.A., M.D. McGill, M.R.C.S.

Observations on the Acidity and Alkalinity of the Blood in Trypanosome Infections. By M. NIERENSTEIN, Ph.D.

Contributions to the Morphology and Life History of *Piroplasma Canis*. By ANTON BREINL, M.U.Dr. Prag., and EDWARD HINDLE, A.R.C.S. Lond. Four plates.

Comparative Chemotherapeutical Study of Atoxyl and Trypanocides. Part I. By M. NIERENSTEIN, Ph.D.

On Three New Species of Culex collected during the Antimalarial Campaign in Mauritius in 1908. M. D'EMMEREZ DE CHARMOY.

Imp. 8vo. Price 7s. 6d. nett

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. II. No. 4.

Concerning the Treatment of Experimental Trypanosomiasis. Part II. By Benjamin Moore, M.A., D.Sc., M. Nierenstein, Ph.D., and John L. Todd, M.D.

An Unusual Case of Goundou. By Dr. R. W. Orpen. One plate.

Sub-Drainage as Applied to the Anti-Malarial Campaign on the Isthmus of Panama. By Henry Simms.

A New Culicid Genus. By F. V. Theobald, M.A.

The Inflicted Talipes of the Chinese. By Frank Jeans, M.A., M.B., B.C. Cantab., F.R.C.S. Eng. Two plates.

Contribution à l'étude de *Porocephalus moniliformis*. Par A. Broden et J. Rodhain.

A New Human Nematode, *Strongylus gibsoni*, n. sp. By J. W. W. Stephens, M.D. Cantab. Two plates.

On the Supposed Occurrence of *Filaria immitis* in Man. By J. W. W. Stephens, M.D. Cantab.

A New Porocephalus (*Porocephalus cercopitheci*, n. sp.). By Anton Breinl, M.U.Dr. Prag., and Edward Hindle, A.R.C.S. Lond.

Comparative Chemo-therapeutical Study of Atoxyl and Trypanocides. Part II. By M. Nierenstein, Ph.D.

Chemical Notes on Atoxyl. By M. Nierenstein, Ph.D.

Note sur le rôle des Tabanides dans la Propagation des Trypanosomiases. Par Le Dr. Edmond Sergent. Imp. 8vo. Price 7s. 6d. nett.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. II. No. 5.

On the Nomenclature of the Mammalian Trypanosomes observed in North Western Rhodesia. By R. Eustace Montgomery, M.R.C.V.S., and Allan Kinghorn, M.B. Toronto.

Experiments on the Combined Atoxyl-Mercury Treatment in Monkeys Infected with *Trypanosoma gambiense*. By Anton Breinl, M.U.Dr. Prag.

Drugs from the Congo. By Prosper H. Marsden, F.C.S.

A Gregarine Parasitic in the Dog-Flea, *Ctenocephalus serraticeps*. By E. H. Ross, M.R.C.S. Eng., L.R.C.P. Lond.

The Action of Aryl-Stibinic Acids in Experimental Trypanosomiasis. By Anton Breinl, M.U.Dr. Prag., and M. Nierenstein, Ph.D. Berne.

Short Note on the Mechanism of Haemolysis in *Piroplasmosis canis*. By Anton Breinl, M.U.Dr. Prag., and H. E. Annett, M.D., D.P.H.

Gland Puncture in the Diagnosis of Animal Trypanosomiasis. By R. Eustace Montgomery, M.R.C.V.S., and Allan Kinghorn, M.B. Toronto.

Observations on the Hooklets of *Cysticercus cellulosae* in Man. By J. W. W. Stephens, M.D. Cantab, D.P.H. Imp. 8vo. Price 7s. 6d. nett

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. III. No. 1.

An Investigation into the Mechanism of Production of Blackwater. By J. O. Wakelin Barratt, M.D., D.Sc., Lond., and Warrington Yorke, M.D., Liverpool.

Imp. 8vo. Price 10s. 6d. nett.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. III. No. 2.

On the Flagellates occurring in the Intestine of *Glossina Palpalis* and in the Intestine and Proboscis of *Glossina Morsitans*. By Allan Kinghorn, M.B., Toronto, and R. Eustace Montgomery, M.R.C.V.S.

Second Report on Human Trypanosomiasis in North-Eastern Rhodesia and Nyasaland. By Allan Kinghorn, M.B., Toronto, and R. Eustace Montgomery, M.R.C.V.S.

A further Report on Trypanosomiasis of Domestic Stock in Northern Rhodesia (North-Eastern Rhodesia). By R. Eustace Montgomery, M.R.C.V.S., and Allan Kinghorn, M.B., Toronto. Two Plates and Map.

Imp. 8vo. Price 7s. 6d. nett.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. III. No. 3.

Sanitary Measures and Malaria Epidemics of Athens. By John Cardamatis. Three plates.

An Account of a Form of Splenomegaly with Hepatic Cirrhosis, Endemic in Egypt. By H. B. Day, M.D., B.S., Lond., M.R.C.P., and A. R. Ferguson, M.D., C.M. Five plates.

Bio-Chemical and Therapeutical Studies on Trypanosomiasis. By Anton Breinl and M. Nierenstein.

Imp. 8vo. Price 7s. 6d. nett.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. III. No. 4.

Reports of the Twenty-first Expedition of the Liverpool School of Tropical Medicine. Jamaica, 1908-1909: Medical and Economic Entomology. Part I.—Ticks and other Blood-sucking Arthropoda. By Robert Newstead, M.Sc., A.L.S., &c. Three plates.

Reports of the Twenty-first Expedition of the Liverpool School of Tropical Medicine. Jamaica, 1908-1909: Malaria. By W. T. Prout, C.M.G., M.B., Ch.M. Three plates.

Imp. 8vo. Price 7s. 6d. nett.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. III. No. 5.

Observations on the Life History of *Trypanosoma lewisi* in the Rat Louse (*Haematopinus Spinulosus*). By Anton Breinl and Edward Hindle. Two plates.

On the Variation of the Haemolytic Complement in Experimental Trypanosomiasis. By Warrington Yorke, M.D.

Acute Craw-Craw. By R. H. Kennan, M.D., D.T.M. Two plates.

A Preliminary Note on the Prevalence of Mosquitoes in Cairo and its Environs. By F. C. Willcocks.

The Effect of Mosquito Larvae upon Drinking Water. By Rubert Boyce, F.R.S., and F. C. Lewis.

Imp. 8vo. Price 7s. 6d. nett.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. IV. No. 1.

The Sanitary Conditions and Diseases Prevailing in Manaos, North Brazil, 1905–1909. By H. Wolferstan Thomas, M.D., C.M. (McGill). Plan and chart.

The Pathological Report of a Case of Œsophagostomiasis in Man. By H. Wolferstan Thomas, M.D., C.M. (McGill). Seven plates.

Etude Zoologique de L'Œsophagostome de Thomas. Par A. Railliet et A. Henry. One plate.

"Mossy Foot" of the Amazon Region, an infective verrucotic condition affecting the skin of the upper and lower limbs. By H. Wolferstan Thomas, M.D., C.M. (McGill). Three plates.

Guarana. By Prosper H. Marsden, F.C.S. One plate.

Some of the Chemical Constituents of Guarana. By M. Nierenstein, Ph.D.

Yellow Fever. By H. Wolferstan Thomas, M.D., C.M. (McGill).

The Mosquitos of the Amazon Region. By R. Newstead, M.Sc., A.L.S., etc., and H. Wolferstan Thomas, M.D., C.M. (McGill). One plate.

Imp. 8vo. Price 10s. 6d. nett

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. IV. No. 2.

Parasitic Granuloma. Conditions allied to Oriental Sore occurring in Egypt. By A. R. Ferguson, M.D., and Owen Richards, F.R.C.S., M.Ch., Oxon. Four plates.

Contribution à L'Étude du *Porocephalus armillatus*. Par A. Broden et J. Rodhain.

On the Absence of a Vesicant in the Ether Extract obtainable from Mosquitos. By J. O. Wakelin Barratt, M.D., D.Sc., London.

Factors in the Transmission and Prevention of Malaria in the Panama Canal Zone. By S. T. Darling, M.D.

Preliminary Experiments on the Effect of Cold on various Diseases in Small Animals. By Professor Major Ronald Ross, C.B., F.R.S., and Major C. L. Williams, I.M.S. (Retired).

Malaria Prevention in Jamaica. By Sir Rubert Boyce, F.R.S. Two plates.

On some Species of *Cyclops* and other Entomostraca collected by Dr. J. M. Dalziel in Northern Nigeria. By G. Stewardson Brady, M.D., LL.D., D.Sc., F.R.S. Three plates.

A New Anopheline from the Federated Malay States. By Malcolm Watson, M.D., D.P.H.

On the Occurrence of Schizogony in an Avian Leucocytozoön, *L. lovati*, Parasitic in the Red Grouse, *Lagopus scoticus*. By H. B. Fantham D.Sc., Lond., B.A., Cantab., A.R.C.S. One plate.

A Case of Sleeping Sickness Studied by Precise Enumerative Methods: Regular Periodical Increase of the Parasites disclosed. By Professor Major Ronald Ross, F.R.S., and David Thomson, M.B., Ch.B., D.P.H.

Imp. 8vo. Price 7s. 6d. nett.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. IV. No. 3.

Some Enumerative Studies on Malarial Fever. By Major Ronald Ross, F.R.S.; and David Thomson, M.B., Ch.B., D.P.H.

A Case of Blackwater Fever followed by a Peculiar Relapse without Haemoglobinuria or Detectable *Plasmodia*. By Major R. Ross, F.R.S.; D. Thomson, M.B., Ch.B., D.P.H.; and G. C. E. Simpson, B.A., B.Sc., F.R.C.S. One chart.

On Haemoglobin Metabolism in Malarial Fever. By G. C. E. Simpson, B.A., B.Sc., F.R.C.S.

Some Observations on a Case of Sleeping Sickness: Coagulation Time of Blood, Albumoses, Choline, Cerebral Sections. By Vishnu T. Korke, M.R.C.P. (Edin.), D.T.M. (Liverpool), L.M.&S. (Bombay).

Some Observations on Malaria in Relation to Splenic Enlargement and the Treatment of the Crescentic Stage. By Dr. N. F. Surveyor, M.A., M.D. (Bom.), M.R.C.P. (Lond.)

On the Peculiar Morphology of a Trypanosome from a Case of Sleeping Sickness and the Possibility of its being a New Species (*T. rhodesiense*). By J. W. W. Stephens, M.D. (Cantab), D.P.H.; and H. B. Fantham, D.Sc. (Lond.), B. A. (Cantab). One plate.

On the Pathogenicity of a Trypanosome (*T. rhodesiense*, Stephens and Fantham) from a Case of Sleeping Sickness Contracted in Rhodesia. By Warrington Yorke, M.D.

On Three New Species of the Genus Glossina, together with a Description of the hitherto Unknown Male of *Glossina grossa*, Bigot. By Robert Newstead, M.Sc., A.L.S., &c.

Descriptions of a New Genus and Three New Species of Anopheline Mosquitos. By R. Newstead, M.Sc., A.L.S., &c.; and H. F. Carter. Two plates

Imp. 8vo. Price 7s. 6d. nett.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. IV. No. 4.

A Note on the Pathology of Lesions of the Cornea and Skin in Animals Experimentally Infected with *T. rhodesiense*. By WARRINGTON YORKE, M.D. Two plates.

A Case of Sleeping Sickness Studied by Precise Enumerative Methods: Further Observations. By Major RONALD ROSS, F.R.S., and DAVID THOMSON, M.B., Ch.B., D.P.H. One chart.

Enumerative Studies on *Trypanosoma gambiense* and *Trypanosoma rhodesiense* in Rats, Guinea-pigs, and Rabbits; Periodic Variations Disclosed. By H. B. FANTHAM, D.Sc., B.A., and J. G. THOMSON, M.A., M.B., Ch.B. Eight charts.

The Life-History of *Trypanosoma gambiense* and *Trypanosoma rhodesiense* as seen in Rats and Guinea-pigs. By H. B. FANTHAM, D.Sc. Lond., B.A. Cantab., A.R.C.S. One plate.

Experiments on the Treatment of Animals Infected with Trypanosomes by means of Atoxyl, Vaccines, Cold, X-rays and Leucocytic Extract; Enumerative Methods Employed. By Major R. ROSS, C.B., F.R.S., and J. G. THOMSON, M.A., M.B., Ch.B.

Auto-agglutination of Red Blood Cells in Trypanosomiasis. By WARRINGTON YORKE, M.D.

On a New Genus of Culicinae from the Amazon Region. By R. NEWSTEAD, M.Sc., &c., and H. F. CARTER.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. V. No. 1.

Further Experimental Researches on the Etiology of Endemic Goitre. By ROBERT MCCARRISON, M.D., R.U.I., M.R.C.P. (Lond.), I.M.S. Two plates.

Non-ulcerating Oriental Sore: the Cultural Characteristics of the Parasite as compared with a New similar Parasite in *Erthesina fullo* (Thumb), a Pentatomid Bug. By Captain R. MARKHAM CARTER, I.M.S. Two plates.

Infantile Leishmaniasis (Marda tal Biccia) in Malta. By A. CRITIEN, M.D., D.P.H., D.T.M. (Liv.)

I.—A Research into the Production, Life and Death of Crescents in Malignant Tertian Malaria, in Treated and Untreated Cases, by an Enumerative Method. By DAVID THOMSON, M.B., Ch.B. (Edin.), D.P.H. (Camb.). Seven charts.

II.—The Leucocytes in Malarial Fever: a Method of Diagnosing Malaria long after it is apparently cured. By DAVID THOMSON, M.B., Ch.B. (Edin.), D.P.H. (Camb.) Eight charts.

Note upon Yellow Fever in the Black Race and its bearing upon the Question of the Endemicity of Yellow Fever in West Africa. By Sir RUBERT BOYCE, F.R.S., M.B. Two charts.

On the Amoebae Parasitic in the Human Intestine, with Remarks on the Life-cycle of *Entamoeba coli* in Cultures. By H. B. FANTHAM D.Sc. Lond., B.A. Cantab., A.R.C.S.

Some Further Observations on the Tsetse-fly, described in these Annals as *Glossina grossa*, etc. By ROBERT NEWSTEAD, M.Sc. A.L.S., Etc.

On the Correlation Between Trypanosomes, Leucocytes, Coagulation Time, Haemoglobin and Specific Gravity of Blood. By VISHNU T. KORKE, M.R.C.P., D.T.M.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. V. No. 2.

Note on Tropical Diseases in Southern Italy. By Professor Umberto Gabbi, (Rome).

The Papataci Flies (*Phlebotomus*) of the Maltese Islands. By R. Newstead, M.Sc., A.L.S., &c. Three plates.

The Experimental Transmission of Goitre from Man to Animals. By Robert McCarrison, M.D., M.R.C.P. (Lond.), I.M.S. Three plates.

Reducing Action of Trypanosomes on Haemoglobin. By Ralph W. Nauss and Warrington Yorke.

The Anti-Malarial Operations at Ismailia. By J. W. W. Stephens, M.D. (Cantab.). Two maps.

On Some New Species of African Mosquitos (*Culicidae*). By R. Newstead, M.Sc., A.L.S., &c., and Henry F. Carter. One plate.

The Diagnosis and Distribution of Human Trypanosomiasis in the Colony and Protectorate of the Gambia. By John L. Todd, M.D., and S. B. Wolbach, M.D. One map.

The Mechanism of the Production of Suppression of Urine in Blackwater Fever. By Warrington Yorke and Ralph W. Nauss. Two plates.

On the Relation of the Organic Phosphorus Content of Various Diets to Diseases of Nutrition, particularly Beri-Beri. By G. C. E. Simpson, B.A., M.B., B.Sc., and E. S. Edie, M.A., B.Sc. Part I.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. V. No. 3.

Tables of Statistical Error. By Professor Sir Ronald Ross, K.C.B., F.R.S., and Walter Stott.

Notes on some Blood Parasites in Reptiles. By Dr. Harald Seidelin. Two plates.

Some Experiments on Larvicides. By Sir R. Ross, K.C.B., F.R.S., and E. S. Edie, M.A., B.Sc.

An Investigation of the Effects produced upon the Excretion of Urinary Pigments by Salts of Quinine. By W. M. Graham, M.B. One plate.

The Passage of Haemoglobin through the Kidneys. By Warrington Yorke, M.D. One plate.

Pseudo-Relapses in cases of Malarial Fever during Continuous Quinine Treatment. By Sir Ronald Ross, K.C.B., F.R.S., and David Thomson, M.B., Ch.B., D.P.H. One chart.

The Trypanosomes found in Two Horses Naturally Infected in the Gambia. By Warrington Yorke and B. Blacklock. One plate.

An Examination of the City of Georgetown, British Guiana, for the Breeding Places of Mosquitos. By K. S. Wise, M.B., B.S., B.Sc., D.P.H. Chart and plate.

A Case of Human Trypanosomiasis in Nyasaland with a Note on the Pathogenic Agent. By Hugh S. Stannus and Warrington Yorke. One plate.

A Second Series of Experiments dealing with the Transmission of Goitre from Man to Animals. By Robert McCarrison, M.D., R.U.I., M.R.C.P. (Lond.) Three plates.

A New Blood-counting Pipette, for estimating the numbers of Leucocytes and Blood Parasites per cubic millimetre. By David Thomson, M.B., Ch.B. (Edin.) D.P.H. (Camb.)

Some Researches on the Life-cycle of Spirochaetes. By H. B. Fantham, D.Sc., B.A., A.R.C.S.

Desmogonius desmogonius, a new Species and Genus of Monostome Flukes. By J. W. W. Stephens, M.D. (Cantab.) One plate.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY. Vol. V. No. 4.

Notes on some Blood-Parasites in Man and Mammals. By HARALD SEIDELIN. Plate XXIV. Two Charts.

The Genus *Pristirhynchomyia*, Brunetti, 1910. By Captain W. S. PATTON, M.B., I.M.S., and Captain F. W. CRAGG, M.D., I.M.S. Plate XXV.

The Life History of *Philaematomyia insignis*, Austen. By Captain W. S. PATTON, M.B. (Edin), I.M.S., and Captain F. W. CRAGG, M.D. (Edin.). I.M.S.

The Measurements of a Thousand Examples of *Trypanosoma vivax*. By B. BLACKLOCK, M.D. Three Charts.

Enumerative Studies on *T. brucei* in Rats and Guinea-Pigs, and a Comparison with *T. rhodesiense* and *T. gambiense*. By JOHN GORDON THOMSON, M.A., M.B., Ch.B. Four Charts.

A Note on the Measurements of *Trypanosoma vivax* in Rabbits and White Rats. By B. BLACKLOCK, M.D.

A Case of Malarial Fever, showing a True Parasitic Relapse, during Vigorous and Continuous Quinine Treatment. By Sir RONALD ROSS, K.C.B., F.R.S., and DAVID THOMSON, M.B., Ch.B., D.P.H. One Chart.

TEXT BOOK

The Practical Study of Malaria and other Blood Parasites. By J. W. W. STEPHENS, M.D., Cantab., D.P.H., and S. R. CHRISTOPHERS, M.B., Vict., I.M.S. Third edition, thoroughly revised, with six coloured plates and one hundred and twenty-eight illustrations in the text 8vo. Price 12s. 6d. nett.

MISCELLANEOUS

Notes on Sanitary Conditions obtaining in Para (1901). By the Members of (1901) THE YELLOW FEVER EXPEDITION. Price 1s.

The Habits of the Marine Mosquito. By Surgeon Ross, R.N. Price 1s.

Mosquito Brigades. By Major R. Ross. GEORGE PHILIP & SON, 32, Fleet Street, London.

The Prevention of Malaria. By Professor R. ROSS, C.B., F.R.S., with contributions by Professor L. O. HOWARD, United States; Colonel W. C. GORGAS, Panama; Mr. J. A. LE PRINCE, Panama; Sir R. BOYCE, West Indies; Dr. W. T. PROUT, Jamaica; Dr. H. WOLFERSTAN THOMAS, Amazon Region; Dr. OSWALDO CRUZ, South Brazil; Dr. IAN MACDONALD, Spain; Professor A. CELLI, Italy; Professor C. SAVAS, Greece; Professor C. SCHILLING, German Possessions; Dr. ED. SERGENT, French Possessions; Mr. H. C. ROSS, Egypt; Dr. A. BALFOUR, Khartoum; Dr. L. BOSTOCK, South Africa; Dr. P. MURISON, Durban; Dr. M. WATSON, Malay States; Professor T. TAKAKI, Formosa; Colonel C. H. MELVILLE, Troops in War; Major C. E. P. FOWLER, Troops in Peace. Published by John Murray & Co., London. Price one guinea.

Prevention of Malaria in the Federated Malay States. By Dr. MALCOLM WATSON, with Preface by Professor R. Ross. Price 7/6. To be obtained on application to the Clerk of the Laboratory, Johnston Tropical Laboratory, University of Liverpool.

The Reduction of Domestic Mosquitos. By Dr. E. H. Ross. Published by John Murray & Co., London. Price 5/-.

Published for

The Committee of the Liverpool School of Tropical Medicine

by

The University Press, 57 Ashton Street, Liverpool

(London Agents: Constable & Co., Ltd., 10 Orange Street, Leicester Square, W.C.)

1912

RC
960
A55
v.5

Annals of tropical medicine
and parasitology

Biological
& Medical
Serials

PLEASE DO NOT REMOVE
CARDS OR SLIPS FROM THIS POCKET

UNIVERSITY OF TORONTO LIBRARY

STORAGE